Distribution of Hydroxyanthraquinones in Soils

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DOCTORAL DISSERTATION

DISTRIBUTION OF HYDROXYANTHRAQUINONES IN SOILS

（土壌中のヒドロキシアンスラキノンの分布）

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CONTENTS

CHAPTER I. INTRODUCTION

Soils are aggregates of water, minerals and organic matter. Soil organic matter is a minor soil component in total soil mass, but is of great importance on soil ecosystem and soil genesis processes.

A kind of a graveyard of the whole terrestrial biosis such as plants and microorganisms, a soil may be expected to contain almost any naturally occurring organic compounds derived from their biosis [Waksman, 1938]. Indeed, many soil organic chemists have researched on such simple organic compounds as amino acids, carbohydrates, lipids, phenols, quinones, as well as on complex humic polymers. Quinone compounds are considered as potential contributors for humuic polymer formations, because these compounds are well known to form stable free radicals [Stevenson, 1982] and presumably Pg fraction of P type humic acids originates from perylenequinone pigments [Kumada, 1987].

Hydroxyanthraquinones are by far the largest group of natural quinones [Thomson, 1971]. They have been found chiefly in higher plants, soil fungi and lichens. Especially, some species of soil fungi may form hydroxyanthraquinones in remarkable abundance. For example, these pigments may constitute up to 30% of dried mycelium of Helminthosporium gramineum, and more than twenty of these pigments have been isolated from

cultures of Penicillium islandicum or other fungal species [Thomson, 1971, 1987]. Therefore, it may be expected that these pigments are incorporated into soil or formed in soil.

Kumada et al. [1961] obtained anthraquinones from the humic acid fraction of volcanic ash soil by alkaline permanganate oxidation. Several investigations for the reductive distillation and fusion products of humic polymers with zinc dust indicated the existence of anthracene together with other polynuclear aromatics [Cheshire et al, 1967, 1968; Hansen and Schnitzer, 1969; Kumada and Matsui, 1970]. In addition, Saiz-Jimenez et al. [1975] reported that hydroxyanthraquinones in fungal metabolites were transformed into dark polymers, and proposed that these process must be occurring in soil. It is possibility that anthraquinone (hydroxyanthraquinone) pigments may be related to the formation of humic substances in soils.

On the other hands, the adjacent OH and O in the hydroxyanthraquinones, which is common chemical structure, may serve as the ligand for polyvalent cations to form complex and participate in translocation (leaching and deposition) of soil mineral, because it is well-known that some of these type compounds give a powerful chelating capacity.

Therefore, research on the behavior and role of hydroxyanthraquinones is a great worthy of note for understanding of soil dynamic system.

A soil anthraquinone pigment was first isolated by McGrath [1967]. The presence of this pigment, a dimer of chrysophanol named as chrysotalunin [McGrath, 1970], was then confirmed in

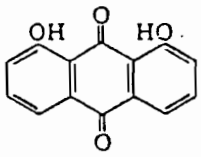
various soils of countries such as Ireland [McGrath, 1967, 1970, 1972], Canada [McGrath, 1972], Japan [Matsui and Kumada, 1974] and New Zealand [Foo and Tate, 1977]. Moreover, McGrath [1972] and Matsui and Kumada [1974] suggest its extremely characteristic distribution. Nevertheless, its behavior and role in soil, and its origin is not completely apparent. On the other hands, regard as the other anthraquinone pigments, there are only a few reports on the occurrence of several compounds (eg. chrysophanol, physcion, etc.) dealing with a very small number of soils [McGrath, 1972; Matsui and Kumada, 1974; Kolesnikov et al., 1978].

The purpose in this paper are to clarify the distribution of soil anthraquinone pigments, in order to dissolved its behavior and role in soils. Therefore, several investigations were made to: (i) research the occurrence of several hydroxyanthraquinone pigments in various soil samples of Hyogo Prefecture, Japan, (ii) develop a method for isolative quantification of chrysotalunin as a most major soil anthraquinone pigment, (iii) determine the relationship between the distribution of chrysotalunin and various soil properties, in a number of Japanese soil, (iv) search the characteristic distribution pattern of chrysotalunin within some soil profiles.

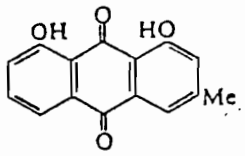
CHAPTER II. SEARCH OF SOIL HYDROXYANTHRAQUINONES

Nearly 600 natural anthraquinones are known and they constitute the largest group of quinon compounds. The great majority are of the conventional polyhydroxy/methoxy type with the usual C_{14} or C_{15} skeleton [Thomson, 1987], and these compounds has been obtained from many soil fungi, higher plants, lichens, etc. Moreover, they have been found in such mineral sources as a burning coal seam and a shale [Thomson, 1971]. In soils, several investigators have also found hydroxyanthraquinones; minute amounts of physcion (**PYS**), chrysophanol (**CPL**), chrysazin (**CZ**), skyrin (**SKY**) and other unidentified polyhydroxyanthraquinones, and relatively great amounts of chrysotalunin (**CLN**), as shown in Fig. II-1.1 [McGrath, 1972; Matsui and Kumada, 1974; Kolesnikov et al., 1978]. Little information is available, however, on their composition in soils.

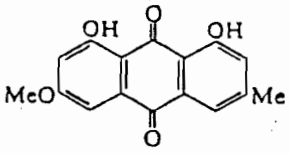
In this chapter, several hydroxyanthraquinone pigments, which have been hitherto detected in soil and in numbers of soil fungi and/or higher plants, were isolated from a soil of the central region of Hyogo Prefecture, Japan (Section II-1). Then, these isolates were identified by spectroscopy and co-chromatography with authentic compounds. Subsequently, a semi-quantitative analysis for the soil hydroxyanthraquinones by thin layer chromatography was conducted to research their composition in some soils of that region (Section II-2).



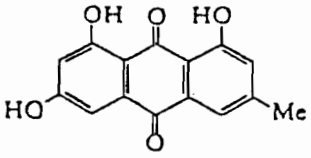
chrysazin



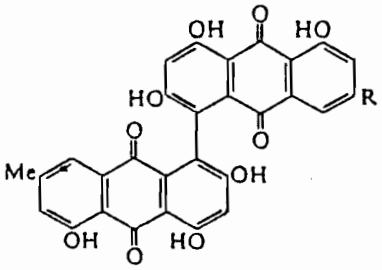
chrysophanol



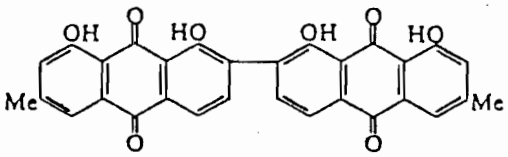
physcion



emodin



R=Me, skyrin
R=CH_2OH, oxyskyrin



chrysotalunin

Fig. II-1.1. Chemical structure of hydroxyantrquinones isolated already from soil.

II-1. Occurrence of Hydroxyanthraquinones in Soils

MATERIALS AND METHODS

Isolation of hydroxyanthraquinones from soils

Seven kilograms of Makino soil sample (HY-14; depth, 0-20cm) in the central region of Hyogo Prefecture, Japan, (See Section II-2, in Table II-2.3) was used for the isolation of the soil hydroxyanthraquinones. The procedure of isolation of their pigments was summarized in Fig. II-1.2.

Five hundred grams of the air-dried soil passed through a 2mm sieve were extracted with hot $CHCl_3$ for two days, and the residue was re-extracted with same procedure.

The $CHCl_3$ solution extracted from the residue was concentrated and then crystalline materials yielded. The crystals (pigment A) were washed with water, acetone, EtOAc and hexane, successively, and recrystallized from $CHCl_3$, repeatedly. About 30mg of pigment A were obtained.

The first $CHCl_3$ extract was shaken with enough 5% NaOH. The NaOH extract was acidified and then extracted with EtOAc. The EtOAc extract was evaporated and chromatographed on silica gel (Silica gel 60, Nakarai Tesque Inc., Japan) by eluting with hexane, benzene and $CHCl_3$, successively. The benzene eluate was evaporated and chromatographed on thin layers of silica gel (Silica gel 60G, Nakarai Tesque Inc., Japan) plate with ace-

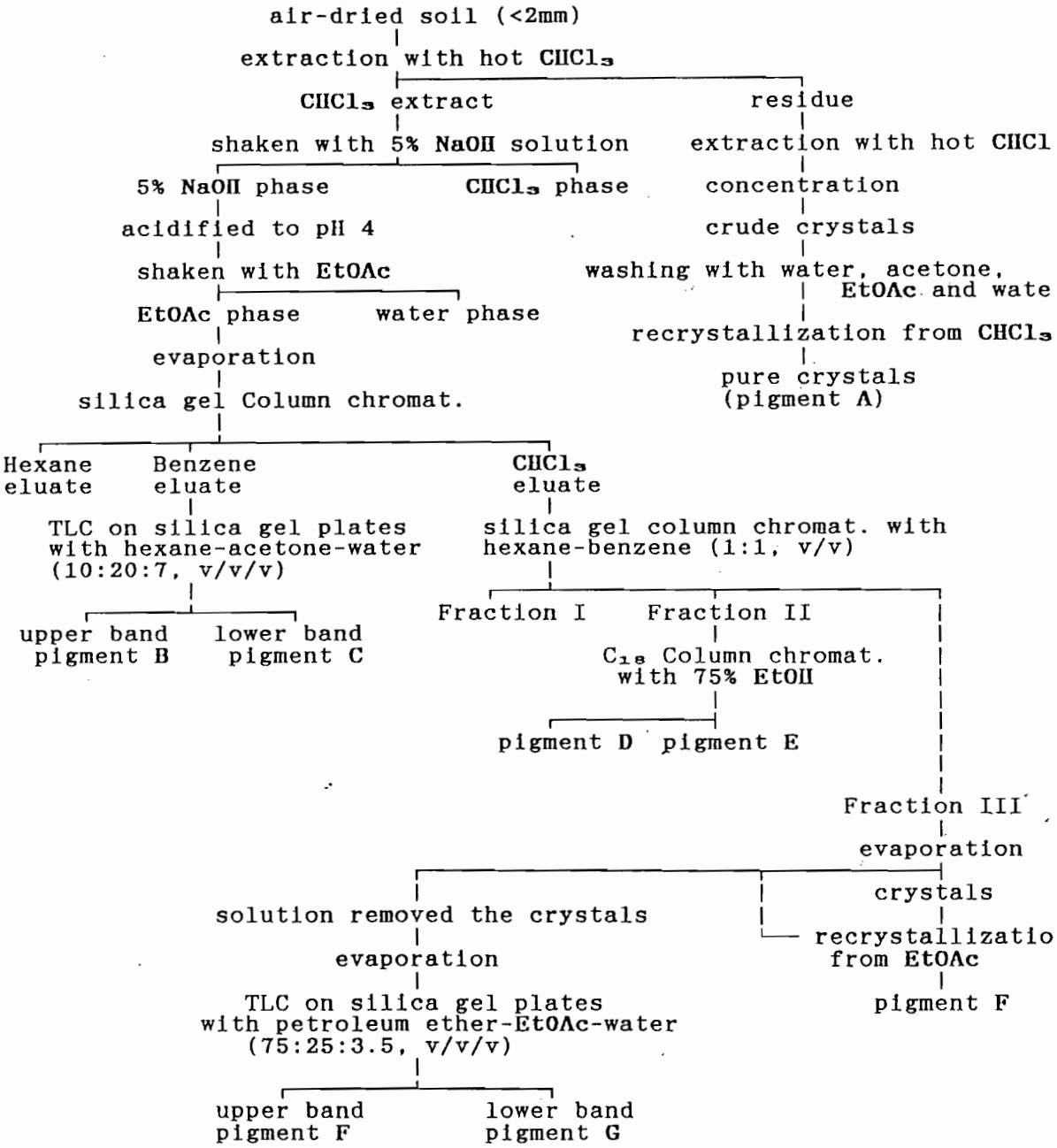


Fig. II-1.2. Procedure of pigment isolation from Makino soil.

tone-hexane-water (10:20:7, v/v/v). Well-separated the upper band (pigment B, <1mg) and the lower band (pigment C, <1mg) were collected and eluted with benzene, respectively. The $CHCl_3$ eluate was evaporated and chromatographed on silica gel. Hexane-benzene (1:1) eluted three yellow bands (Fraction I, II and III). The fraction II was evaporated and re-chromatographed on a column of Cosmosil $75C_{18}$-OPN (Nakarai Tesque Inc., Japan). Ethanol-water (1:3) eluted two well-separated yellow bands (pigment D and E, <1mg). A crystalline pigment F obtained from concentrate of the fraction III was purified by repeatedly recrystallization from EtOAc. The EtOAc solution removing the crystals was chromatographed on thin layer of silica gel plate described above with petroleum ether (b.p.50-80°C)-EtOAc-water (75:25:3.5, v/v/v) [Ma et al., 1989]. The upper band (pigment F) and the lower band (pigment G) were respectively collected and eluted with $CHCl_3$. As results, 7mg of pigment F and small amounts (<1mg) of pigment G were obtained.

Analyses of isolates

The isolated pigment A - G were developed on thin layer silica gel plates (TLC aluminum sheets Silica gel 60 F_{254}, E. Merck, Darmstadt), using the each following solvent: A) benezen-ethylformate-HCOOH, (75:24:1, v/v/v) [Leistner, 1971], B) dibutylether-CH_3COOH (10:1, v/v) [Leistner, 1971] , C) hexane-acetone-water (10:10:7, v/v/v) [Furutani, 1958], D) petroleum

ether (b.p.50-80°C)-EtOAc-water (75:25: 3.5, v/v/v) [Ma et al., 1989], and E) benzene-methanol (4:1, v/v) [Rai and Shok, 1981].

Five authentic reagents of anthraquinones — CZ, CPL, PYS, emodin (EMD) and SKY, were co-chromatographed with these unknown pigments. Each spot was observed under UV-light and then made an examination of specific color reactions by spraying with alcoholic KOH solution. The mass spectra of electron impact method (EI/MS) were measured on a Hitachi Model 6-MG mass spectrometer with direct inlet at 70eV. The ultraviolet and visible spectra (UV-VIS) were measured on a Hitachi 200-10 spectrometer.

For some of the pigment, the high resolution MS were measured on a Hitachi Model 80A double focus MS spectrometer with direct inlet at 70eV, and the reaction gas for the mass spectra of chemical ionization method (CI/MS) was isobutane. NMR spectra were measured on a Brucker AC type 250MHz NMR spectrometer.

RESULTS AND DISCUSSION

Isolation and identification of pigments

For isolated pigments and authentic reagents, their R_f values and specific color reaction on thin layer chromatography, their maximum peaks of UV-VIS, and EI/MS data were shown

in Tables II-1.1, II-1.2 and II-1.3, respectively.

Generally, hydroxyanthraquinone pigments indicates positive color reaction by KOH-alcoholic solution and indicates maximum peaks at 240-260 (benzenoid band), 260-290 (quinoid band) and 400-500nm by UV-VIS [Thomson, 1971]. As shown in Tables II-1.1 and II-1.2, all pigments isolated indicated positive properties for hydroxyanthraquinone pigments.

In table II-1.1, solvent system (C) gave a good separation of CPL, PYS and other authentic pigments. As shown in this

Table II-1.1. Characteristics of isolated pigment A - E and authentic samples on thin layer chromatography.

compounds	R_f values*					color reaction with 5% alcoholic KOH solution
	(A)	(B)	(C)	(D)	(E)	
pigment A	0.83	0.00	0.00	0.00	0.81	purple
B	0.83	0.83	0.64	0.65	0.77	red
C	0.83	0.76	0.55	0.63	0.77	red
D	0.86	0.69	0.33	0.53	0.81	purple
E	0.85	0.61	0.28	0.44	0.81	red
F	0.85	0.52	0.22	0.35	0.82	purple
G	0.37	0.60	0.15	0.25	0.43	red
chrysazin	0.80	0.83	0.59	0.59	0.76	red
chrysophanol	0.83	0.83	0.64	0.65	0.77	red
physcion	0.83	0.77	0.55	0.63	0.77	red
emodin	0.37	0.61	0.15	0.25	0.43	red
skyrin	0.14	0.45	0.01	0.05	0.22	purple

* solvent systems: A) benzene-ethyl formate-HCOOH (75:24:1,v/v/v); B) dibutylether-CH_3COOH (10:1, v/v); C) hexane-acetone-water (10:10:7, v/v/v); D) petroleum ether (b.p.50-80°C)-EtOAc-water (75:25:3.5, v/v/v); E) benzene-MtOH (4:1, v/v).

Table II-1.2. UV-VIS absorption of isolated pigment A - E and authentic samples.

compounds	solvent	$\lambda_{max.}$ nm*				
pigment A	$CHCl_3$		264	289		440-460
B	EtOH	256	276	286		429
C	EtOH	257	265	288		430
D	EtOH	248	263			438 450
E	EtOH	230	260			432 455
F	$CHCl_3$	246	270	286	310	444 460
G	EtOH	255	267	291		435
chrysazin	EtOH	251	273	283		429
chrysophanol	EtOH	257	277	287		429
physcion	EtOH	257	266	288		431
emodin	EtOH	253	266	289		436
skyrin	DiOx	258		290		448

* The data with under line indicates shoulder peaks.

Table II-1.3. EI/Mass spectra of isolated pigment A - E and authentic samples.

compounds	M^+ (%)	Principal ions (%)
pigment A	506(100)	489(36), 460(6), 431(5), 253(12)
B	254(40)	238(100), 210(5), 181(13)
C	284(100)	255(5), 241(4), 213(3), 185(6)
D	638(0.5)	623(7), 385(100), 371(8)
E	not detectable	
F	566(100)	549(8), 535(99), 520(6), 517(7), 506(5), 491(8), 297(48), 283(15), 267(8), 262(7)
G	270(100)	242(7), 241(7), 213(4)
chrysazin	240(100)	212(13), 184(12)
chrysophanol	254(44)	238(100), 226(5), 210(8), 181(19), 152(18)
physcion	284(100)	255(7), 241(6), 226(3), 213(4), 185(3)
emodin	270(100)	242(5), 241(6), 213(5), 185(2), 135(5)
skyrin	not detectable	

table, The R_f values and color reactions of pigment B, C and G were correspondent with those of CPL, PYS and EMD, respectively. These three pigments, B, C and G were also confirmed to be CPL, PYS and EMD, respectively, by the results of UV-Vis and EI/MS (Tables II-1.2 and II-1.3). On the other hands, the results in these experimental data of pigment A, D, E and F were not in agreement with the data of the authentic samples of known compounds in soil and of several famous compounds as metabolites of plants and/or fungi.

The pigment A was insoluble in common organic solvents and sparingly soluble in $CHCl_3$. The UV-VIS and the EI/MS were shown in Figs. II-1.3a and II-1.4a, respectively. The CI/MS showed a base peak at $_{M/Z}$ 505. The EI/MS of its acetate treated with pyridine and acetic anhidride showed a very weak and highest peak at $_{M/Z}$ 674 and four particular peaks suggesting the stepwise loss of acetyl ($_{M/Z}$ 42) units (Fig. II-1.4b). These results are consistent with the data of CLN published by McGrath [1970] and Foo and Tate [1977]. The NMR spectrum of its acetate is identical with data of their reports (Table II-1.4). Therefore, the pigment A was identified as CLN.

The pigment F was estimated as hydroxyanthraquinones by indicating UV-VIS spectra (Fig. II-1.3b) and positive Mg(OAc) and alkali tests [Thomson, 1971]. The EI/MS and the data of the high resolution MS of significant fragment ions were shown in Fig. II-1.4c and Table II-1.5, respectively. The CI/MS showed a base peak at $_{M/Z}$ 567. The EI/MS of trimethylsilyl ether prepared by treatment [Van Eijk and Roeijmans, 1984] of

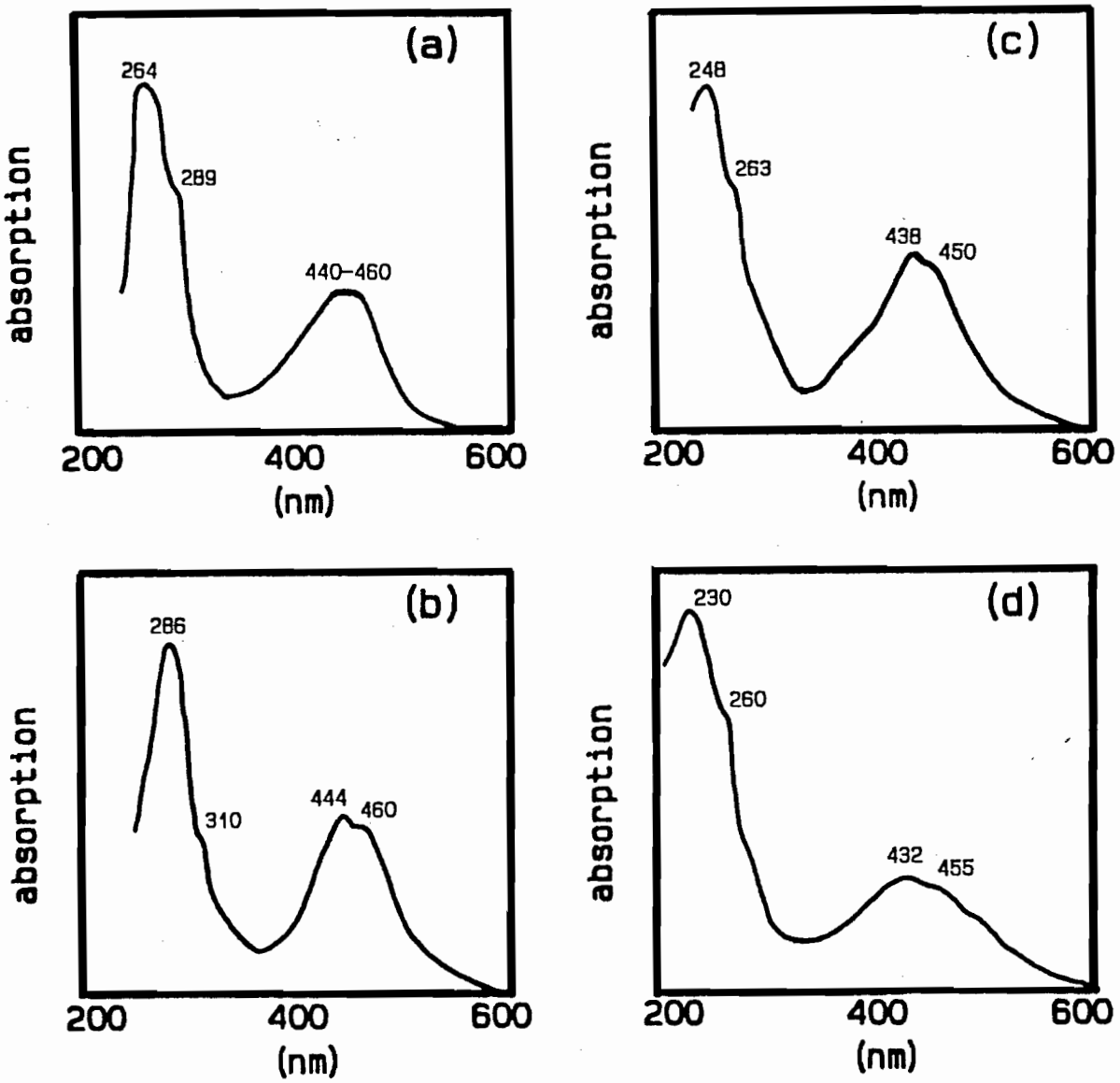


Fig. II-1.3. UV-VIS absorption spectra of some isolated pigments; a) pigment A (in $CHCl_3$), b) pigment F (in $CHCl_3$), c) pigment D (in EtOH), and d) pigment E (in EtOH).

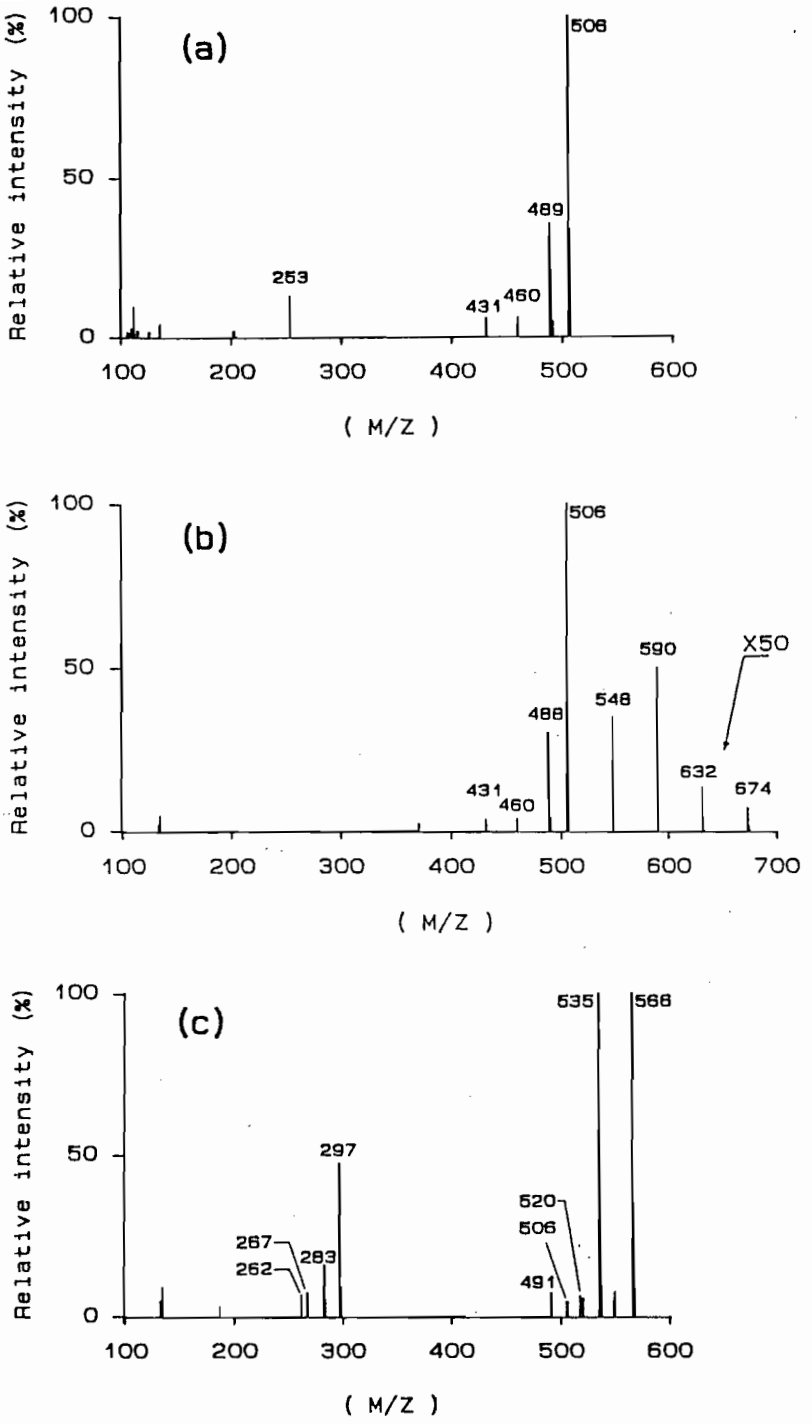


Fig. II-1.4(a-c). EI/MS spectra of some isolated pigments; a) pigment A, b) pigment A-acetate, c) pigment F.

Table II-1.4. ^{1}NMR spectra of peracetylated pigment A and E in $CDCl_3$.

Peracetyl derivative of	Chemical shifts, δ 2-H	4-H	5-H	6-H	O-Me	C-Me
Pigment A	7.25_m	8.05_m	8.27_d	$7.70[6H]_d$		2.52_t 2.19_s 2.42_s
Pigment E	7.22*	8.04*	7.76_s		3.93_s	2.12_t 2.40_s 2.50_s

* broad singlet
The marks of subscripts indicates as follows; m, multiplet; t, triplet; d, doublet; s, singlet.

Table II-1.5. The Data of high resolution MS of pigment E.

[M/Z]	obsd.	calcd.	composition
M^+	566.1205	566.1210	$C_{32}H_{22}O_{10}$
M-31	535.1053	535.1027	$C_{31}H_{19}O_9$
M-61	505.0936	505.0922	$C_{30}H_{17}O_8$
M-269	297.0720	297.0678	$C_{17}H_{13}O_5$
M-283	283.0607	283.0606	$C_{16}H_{11}O_5$

the pigment showed a highest peak at $_{M/Z}$ 854, suggesting that the parent compound contained four OH groups. Thus, it was presumed that the pigment F is tetra-hydroxy methoxy bianthraquinone derivative. These results are consistent with the spectral data of 7,7'-biphyscion (BP) (Fig. II-1.5) [Gluchoff et al., 1972; Stegrich et al., 1972]. The ^{1}H NMR-spectrum of acetylated product of the pigment F (Table II-1.4) is also identical with the reported NMR-spectrum for BP tetra-acetate [Stegrich et al., 1972]. Therefore, the pigment F was identified as BP.

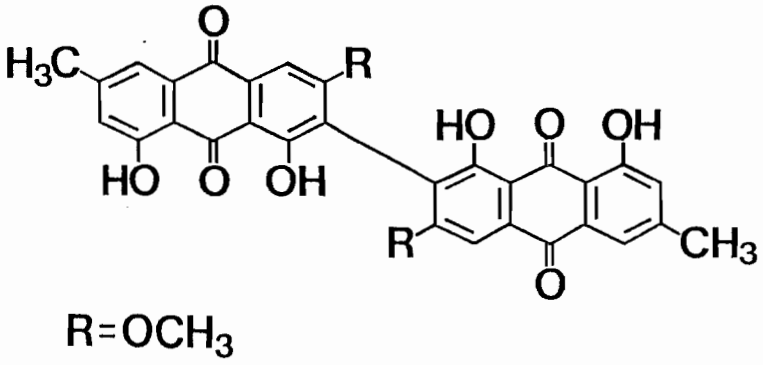


Fig. II-1.5. Chemical structure of 7,7'-biphyscion (pigment F).

The **UV-VIS** of the pigment **D** was shown in Fig. II-1.3c. The **EI/MS** of the pigment **D** and its acetate were shown in Fig. II-1.6. This pigment also gave positive properties for hydroxyanthraquinones by **Mg(OAc)** and alkali tests. This pigment was also estimated to be hydroxyanthraquinones by the figure of **UV-VIS** spectra. In the figures of the **MS** fragments, a highest peak at 638 (Fig. II-1.6a) and principal peaks at 632, 590, 548 and 506 (Fig. II-1.6b) suggested that the parent pigment might be relative compound of **CLN** such as its glycoside. However, amount of the **D** pigment was not enough to carry out other spectral analyses (cf. **NMR**).

The **EI/MS** of the pigment **E** is not detectable because of its possible unvolatilic property. However, this pigment was estimated as hydroxyanthraquinones by indicating **UV-VIS** spectra (Fig. II-1.3d) and positive **Mg(OAc)** and alkali tests [Thomson, 1971]. Since this pigment could not be obtained enough amounts

for other spectral analyses, as well as pigment **D**, its chemical structure is unknown.

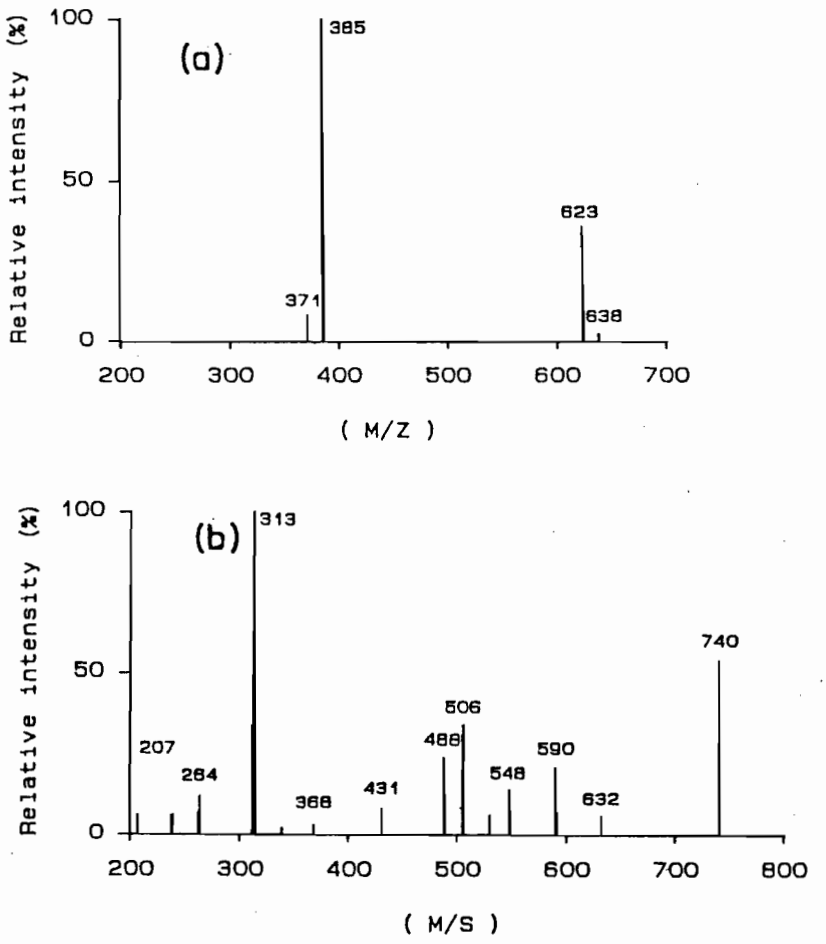


Fig. II-1.6. EI/MS spectra of some isolated pigments; a) pigment **D** and b) pigment **D**-acetate.

Occurrence of pigments in Makino soil

In this experiments, seven of hydroxyanthraquinone pigments were isolated from Makino soil and five of those were identified as chrysophanol, physcion, emodin, chrysotalunin, 7,7'-biphyscion.

CLN is a first isolated quinone compound from soil by McGrath [1967]. It is well-known that this pigment is a most prominent anthraquinone in soil and has now been found in soils from Ireland, Canada, New Zealand, and Japan [McGrath, 1970, 1972; Matsui and Kumada, 1974, Foo and Tate, 1977]. Indeed, **CLN** also gave the largest amount of all pigment isolated in this experiments. **CPL** and **PYS** are common compounds in nature — they distributed widely in moulds, lichens, higher plants, especially in a number of soil fungi [Thomson, 1987]. They also have been found in a few soils of Ireland and Japan and were accompanied by large amount of **CLN** [McGrath, 1972; Matsui and Kumada, 1974]. These facts suggest that these three pigments are provably common compounds in soils.

EMD and **BP** were first compounds isolated from soils. **EMD** is probably one of the most major compound in naturally occurring anthraquinones because a large proportion of natural anthraquinones are biosynthetic variations of this basic structure and it has a most wide distribution in nature [Thomson, 1971]. However, **EMD** obtained from Makino soil is the smallest amount in these seven pigments, and surprisingly, some investigators studied on soil anthraquinone pigments did not find it. On the other hands, **BP** has so far been isolated from sporo-

phores of two higher fungi, Dermocybe cinnamomeolutea [Gluchoff et al., 1972] and Tricholoma equestre [Stegrich et al., 1972]. BP was the next higher amounts in the pigments obtained from Makino soil. Nevertheless, there are no literature on isolation of the pigment from other biological materials, of course from soils.

To clarify the origin of these soil pigments, some fungi were isolated from this soil by the soil plate dilution technique. Their mycelia incubated on Martin's medium [Martin, 1950] and their media were examined for occurrence of anthraquinones. Although only CPL-containing fungi were obtained, other dimer or monomer pigments were not apparent. These findings as described above may depend on the growth conditions and the difficulties in isolation of soil fungi, or the presence of other microbial origin, especially actinomycetes. The overlying vegetations of Makino soil were Miscanthus sinensis and Sasa spp. in which occurrence of hydroxyanthraquinones has yet not been demonstrated. Although many anthraquinones have been found in higher plants, their distribution are limited in some peculiar kinds of plants [Thomson, 1987]. Therefore, it seems that the origin of these soil pigments may be not plant materials but microorganisms.

The results in this experiments are not in accord with such expectation that major naturally occurring pigments (chrysophanol, physcion, especially emodin) may be predominant in soils. BP is a kind of CLN derivative in other words. Moreover, the

pigment D also may be CLN derivative although its detailed chemical structure could not established. While relation among CLN and these compounds is unknown, the fact that these similar compounds are major in this soil is interest for investigation of soil anthraquinone pigments.

II-2. Composition of Hydroxyanthraquinones in Soils

MATERIALS AND METHODS

Soil Samples

Soil samples were randomly taken from the surface horizons (A horizons) of four brown forest soils and four ando soils, in the central region of Hyogo Prefecture, Japan. The characteristics of the soils are given in Table II-2.1.

Table II-2.1. Soil samples used.

Plot Nos.	Soil name	Soil* types	pH (H_2O)	T-C** (%)	principal vegetations
HY-3	Yamanobe	B_B	4.1	7.2	Chamaecyparis
HY-4	Isarigami	B_C	3.6	4.3	Chamaecyparis
HY-6	Mitani	B_D	4.0	7.8	Chamaecyparis
HY-10	Maya	B_D	4.1	5.4	Castanopsis
HY-12	Mineyama	Bl	4.5	10.9	Cryptomeria, Sasa
HY-13	Sugyome	Bl	4.6	7.3	Chamaecyparis
HY-14	Makino	Bl	4.6	12.2	Sasa, Miscanthus
HY-17	Mimuro	Bl	5.1	8.9	Cryptomeria, Sasa
HY-19	Saiki	B_D	5.2	2.1	Chamaecyparis, Sasa
HY-20	Hachiura	B_D	5.3	3.9	Cryptomeria

* According to Forest Soil Division [1976].
** total carbon.

Detection of soil hydroxyanthraquinones

A soil sample (30g) was shaken with a mixed solvent of acetone-2N **NaOH** (1:1, v/v) as described by McGrath [1972]. Total acetone extracts were added distilled water, acidified and extracted with **EtOAc** in a separatory funnel. The **EtOAc** extract was shaken with enough 5% **NaOH**. The **NaOH** extract was re-extracted with **EtOAc**. **EtOAc** extracts were washed with enough distilled water and then evaporated. The residue was chromatographed on silica gel by eluting with hexane-**EtOAc** (3:1, v/v). The obtained eluate was evaporated and the residue was dissolved in $CHCl_3$. A portion of the solution was spotted on thin layer silica gel plates (10cmX10cm, TLC aluminum sheets Silica gel 60 F_{254}, E. Merck, Darmstadt) by micropipet for **TLC** and chromatographed in two directions with (1) benzene-ethylformate-**HCOOH** (75:24:1, v/v/v) [Leistner, 1971] in the first direction and with (2) hexane-acetone-water (10:10:7, v/v/v) [Furutani, 1958] in the second direction. The spots were detected by spraying alcoholic **KOH** solution.

RESULTS AND DISCUSSION

Thin layer chromatograms of soil extracts

The two developing solvent systems which were examined in Section II-1 were applied to the thin layer chromatography for

anthraquinones extracted from each soil sample. As shown in Fig. II-2.1(a-k), their thin layer chromatograms gave well separation of several hydroxyanthraquinone pigments in these soils. However, this experiments with the mixture of the authentic samples indicate that the separation of CPL and CZ is not suitable. Therefore, to obtain the EI/MS of the doubtful spot, a portion of each soil extract was supplied on silica gel layer chromatography (1mm, 20X20cm) with the solvent system (2). As the results, all doubtful spots were confirmed to be CPL. Only one extracts from Mimuro soil sample (HY-17) gave a trace another positive spot ($_{M/z}$ 240, possible CZ) for hydroxyanthraquinones with the spot of CPL, altough its presence was not detectable level in normal method.

On the separation and determination of anthraquinones, thin layer chromatography is preferable to other chromatographic methods for many reasons. For example, although Van Eijk and Roeijmans [1984] reported good separation of a mixture of various trimethylsilylated hydroxyanthraquinones by capillary gas chromatography, low responses of these pigments are not enough to determine of these soil pigments. Thin layer chromatography in this experiments gave good separation of these soil pigments. It is well-known that the dimensions of the spots of anthraquinone pigments on plates were approximately proportional to its concentration. Only CLN cannot be detected quantitatively, because of its extremely low solubility and its strong adsorption on surface of silica gel and glassware. Thus, most

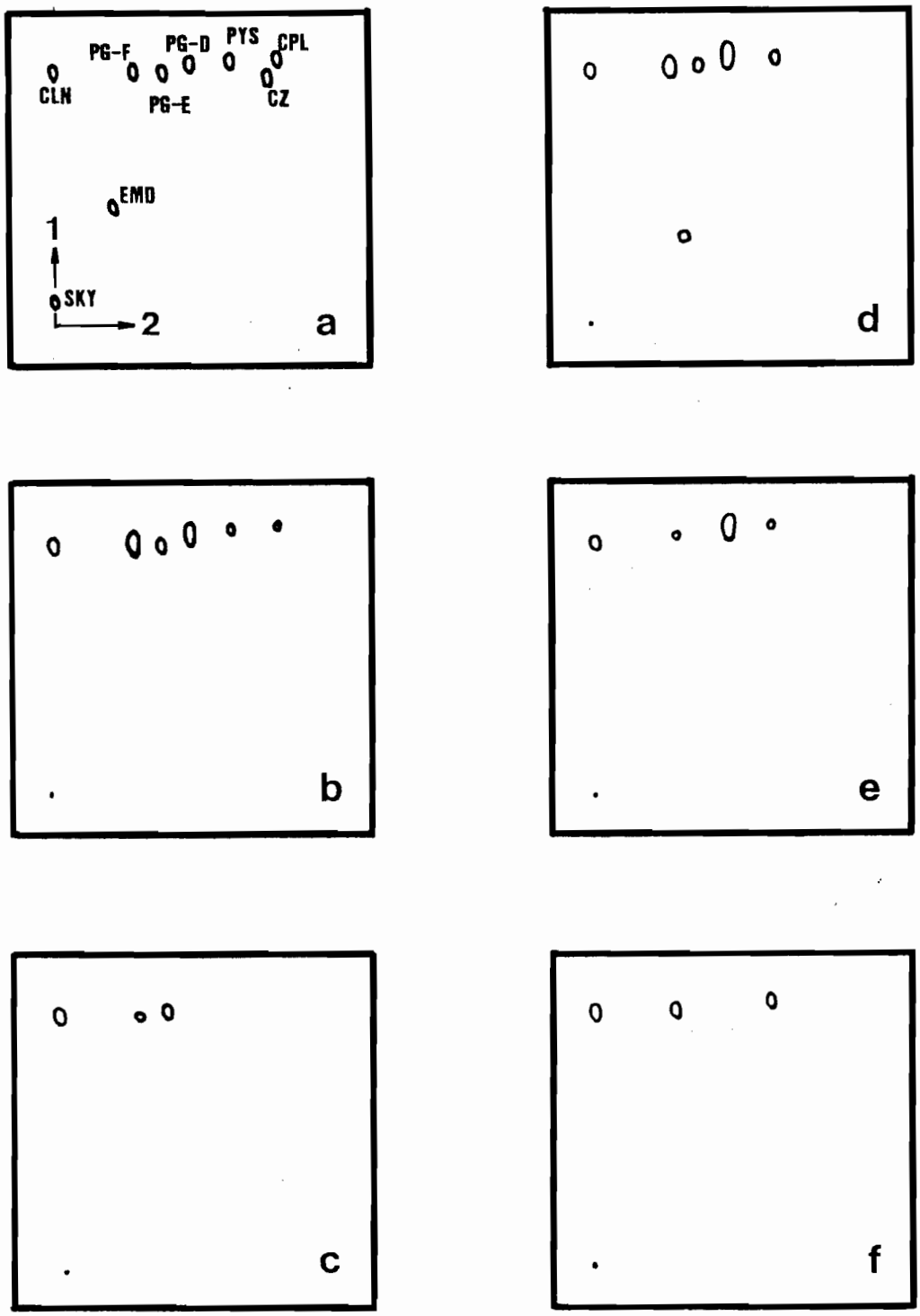


Fig. II-2.1(a-f). Thin layer chromatograms of hydroxyanthraquinones from soils; a) standard b) HY-3, c) HY-4, d) HY-6, e) HY-10, and f) HY-12.

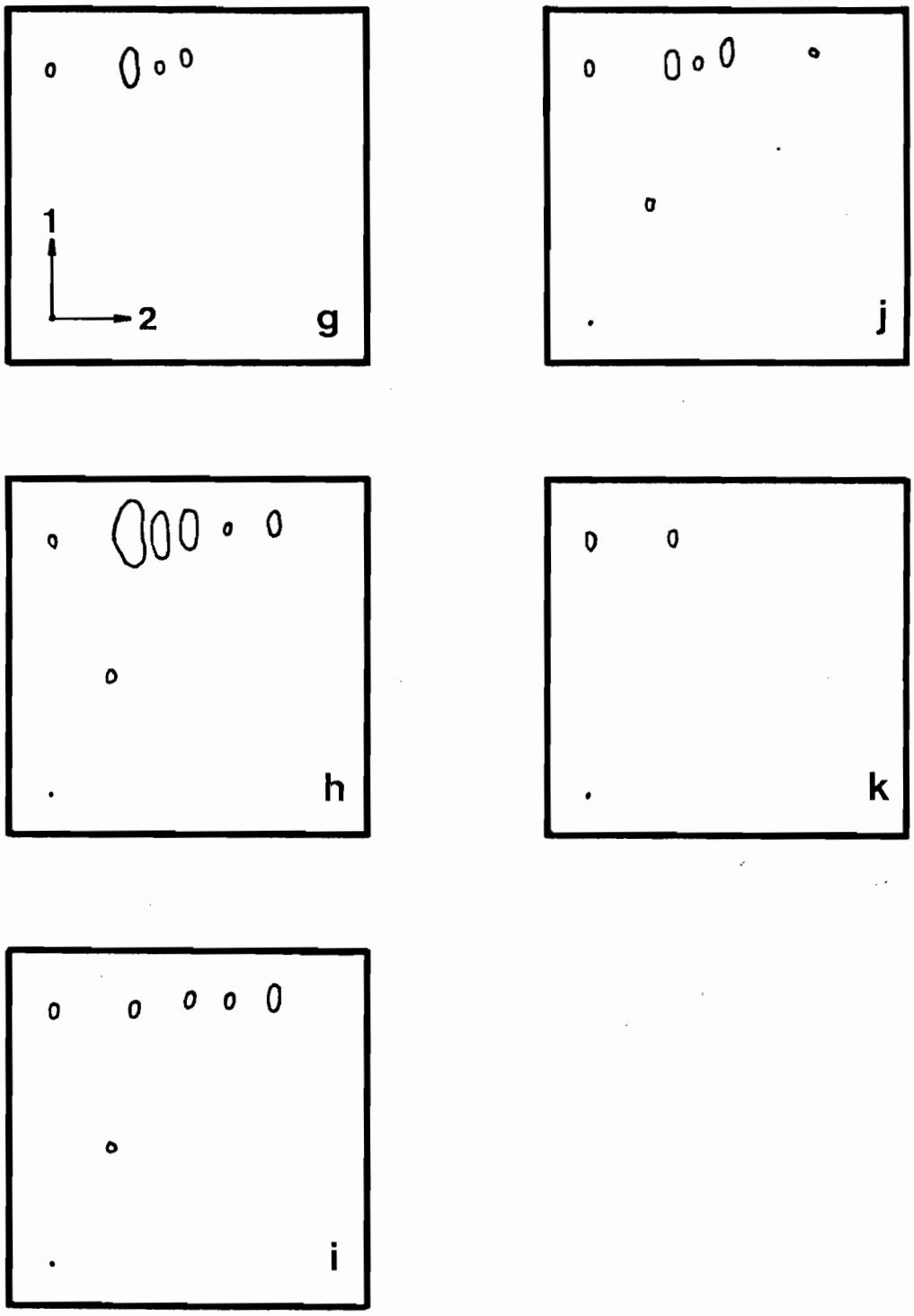


Fig. II-2.1(g-k). Thin layer chromatograms of hydroxyanthra quinones from soils; g) HY-13, h) HY-14, i) HY-17, j) HY-19, and k) HY-20.

of **CLN** was remained on column of silica gel in this experimental procedure. However, since its content is usually ten times to a hundred or more times as high as the contents of other pigments in soils, it was possible to confirm the presence of **CLN** therein.

Distribution of pigments in soils

Table II-2.2 shows the composition and distribution of hydroxyanthraquinone pigments in various soils. All soil samples contained from two to seven of hydroxyanthraquinone pigments.

According to Matsui and Kumada, they confirmed the presence of **CLN**, **CPL**, **CZ**, **PYS** and **SKY** in a black soil of Japan. In this experiments, **CZ** and **SKY** were not found in four black soils and six brown forest soils.

Table II-2.2. Distribution of hydroxyanthraquinones in soils

Plot Nos.	CLN (PG-A)	CZ	CPL (PG-B)	PYS (PG-C)	(PG-D)	(PG-E)	BP (PG-F)	EMD (PG-G)	SKY
HY-3	+		±	±	++	+	++		
HY-4	+					+	±		
HY-6	+			+	++	+	++		
HY-10	+			±	++		±		
HY-12	+			+			+		
HY-13	+				+	+	+++		
HY-14	+		++	+	+++	+++	+++++	+	
HY-17	+		++	+	+		+	+	
HY-19	+		±		++	+	++	+	
HY-20	+						+		

Spots size (max. diameter X min. diameter, mm); ±, <5; +, 5-10; ++, 10-20; +++, 20-30; +++++, >50

CPL, PYS and EMD, namely monomer compounds, were randomly detected in four, six and three soil samples, respectively. On the other hands, the dimer compounds such as CLN and BP were detected in all soil samples. Moreover, possible dimer compound, the pigment D were also detected in seven soil samples. Although semi-quantitative method was used in this experiments, it is clear that these dimer compounds are quantitatively predominant in these soils. Although BP had been isolated from two higher fungi [Stegrich et al., 1972; Gluchoff et al., 1972], no other producer has yet been found. Moreover, the origin of CLN is uncertain, although it appears most likely to be a fungal metabolite [McGrath, 1970, 1972].

While a great number of anthraquinones have been now found in various biosis [Thomson, 1987], limited compounds has been detected in some soils [McGrath, 1972; Matsui and Kumada, 1974; Kolesnikov et al. 1978]. Since monomer compounds in soils were very common compounds in anthraquinone groups, these pigments must be occurring in soils. However, most prominent soil pigments were dimer compounds. It seems curious that the pigments actually detected in soils are very few with respect to kind and quantity.

In this experiments, soil samples containing various amounts of carbon under the different vegetations were used. No obvious relation between the presence of soil pigments and soil types, organic carbon contents or principal vegetations are apparent as shown in Tables II-2.1 and II-2.2.

The occurrence of the organic compounds in the free state in soils may be influenced on their biodegradability, activity (or productivity) of their biological origin, and their reactivity on soil minerals or humic polymers. McGrath [1972] suggested that the CPL and PYS namely monomers were probably of transient occurrence. Linhares and Martin [1979] reported that CPL and EMD were quickly degraded in a certain soil, percentage of decomposition at 12 weeks being 69 and 47%, respectively. However, the facts that these similar biantharquinones are predominant in soils is of particular interest for investigation of the behavior of soil anthraquinone pigments.

In this experiments, the accurate concentrations of individual compounds were not obtained. This method will permit the densitometric determination of individual soil hydroxyanthraquinones by studying several calculative condition, except for CLN. Further studies is need for search of the soil pigments.

CHAPTER III. DISTRIBUTION OF CHRYSOTALUNIN IN SOILS

In Chapter II, of particular interest is predominantly occurrence of bianthraquinones, such as chrysotalunin and 7,7'-biphyscion, in soils.

Chrysotalunin (**CLN**, Fig. III-1.1), a dimer of 1,8-dihydroxy-3-methylanthraquinon is the first quinone from soil, and its world-wide distribution and its much higher accumulation in soils comparing with other soil anthraquinones is well-known [McGrath, 1967, 1970, 1972; Matsui and Kumada, 1974; Foo and Tate, 1977].

Moreover, McGrath [1972] and Matsui and Kumada [1974], suggested that the distribution of **CLN** in soils might have some characteristic pattern. There is, however, only limited information on its distribution in soils. Chemical properties of **CLN**, such as its extremely low solubility and its strong

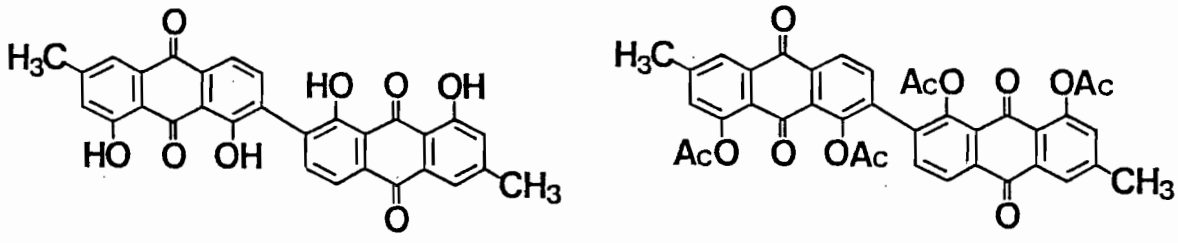


Fig. III-1.1. Chemical structure of **CLN** and its tetraacetate.

adsorption on silica gel and glassware, frequently obstruct its quantitative isolation. McGrath's method for determination of **CLN** in soils using simple colorimetry may be frequently influenced by other organic compounds in soils. To clarify the detailed distribution of **CLN** in various soils, it is, therefore, necessary to establish the isolative determination methods useful for **CLN** in soils.

The purpose in this chapter is to develop a isolative determination for the reliable estimation of **CLN** in soils and subsequently to study the characteristics of distribution of **CLN** in soils by the new method developed.

In Section III-1, the new method based on the acetylation of **CLN**, and separation and determination of CLN tetraacetate (**CLN-Ac**, Fig. III-1.1) by liquid chromatography was proposed. Next, in Section III-2, it was studied on the geographical distribution of **CLN** in various surface soils taken from wide locations of Japan and difference of its distribution in several groups of soils. Subsequently, in Section III-3, the distribution patterns of chrysotalunin within several soil profiles were examined.

III-1. Determination Method for Chrysotalunin in Soils

MATERIALS AND METHODS

Soil samples

Several characteristics of soils used are given in Table III-1.1. These soils were taken from the A horizons at seventeen locations in Hyogo Prefecture, Japan. Each soil was air-dried and passed through a 2mm screen. A portion of the sample was ground to pass a 0.2mm screen for the following analysis.

Table III-1.1. Soil samples used.

Plot Nos.	Soil name	Depth (cm)	Munsell notation	T-C* (%)	pH (H_2O)
		< Red soils>			
HY-1	Azumi	0-20	7.5YR 3/4	2.7	4.4
		<Brown forest soils>			
HY-2	Yamada	0-20	10 YR 2/3	3.5	4.9
HY-3	Yamanobe	0-5	10 YR 3/4	7.2	4.1
HY-4	Isarigami	0-25	7.5YR 3/2	4.3	3.6
HY-5	Todoroki	0-22	10 YR 3/4	3.6	4.1
HY-6	Mitani	0-17	10 YR 2/1	7.8	4.0
HY-7	Ohyama I	0-5	7.5YR 4/4	12.6	4.7
HY-10	Maya	0-40	7.5YR 2/2	5.4	4.1
HY-11	Ohyama II	0-8	7.5YR 2/2	8.9	4.3
HY-21	Saiki	0-22	10 YR 3/3	2.1	5.2
		<Black soils>			
HY-12	Mineyama	0-20	5 YR 1.7/1	10.9	4.5
HY-13	Sugyoume	0-10	7.5YR 2/2	7.3	4.6
HY-14	Makino	0-40	7.5YR 1.7/1	12.2	4.6
HY-15	Hachikita	0-20	5 YR 1.7/1	15.4	5.5
HY-16	Hachibuse	0-20	7.5YR 1.7/1	17.6	5.7
HY-17	Mimuro	0-20	7.5YR 1.7/1	8.9	5.1
HY-18	Kannabe	0-20	7.5YR 1.7/1	18.0	5.5

* T-C is total organic carbon and was determined by rapid dichromate oxidation method [Page et al, 1982].

Spectrometric analysis

Ultraviolet and visible (UV-VIS) spectra were obtained on a Hitachi 200-10 spectrometer. The mass (MS) spectrometric analyses were performed on a Hitachi Model 80A double focus MS spectrometer. The ionization voltage was 70eV for electron impact (EI) and chemical ionization (CI) method. The reaction gas of CI was isobutane. A sample was directly inserted into the ion chamber through a heated inlet system at 200°C.

Preparation of chrysotalunin for standardization

A standard of pure CLN was obtained from Makino soil (Table III-1.1) for preparing a calibration curve. Five kg of the air-dried soil were extracted with a 1:1 mixed solvent of acetone and 2N NaOH, as described by McGrath [1972]. The upper (acetone) layer was acidified and the resulting precipitate was collected by centrifugation. The precipitate was repeatedly washed with distilled water, EtOH and EtOAc until the washings were no longer colored, and re-extracted with $CHCl_3$ in a Soxhlet apparatus. A crystalline orange pigment obtained from the concentrated extract was purified by repeated recrystallization from chloroform. Seventy milligrams of pure crystals were obtained. It was identified by comparing the solubility in various solvents, the color reaction, the UV-VIS absorption spectra and the EI/ and CI/MS spectra of the purified pigment with those published for CLN [McGrath, 1970], as described in Section II-1.

The CLN standard was acetylated with pyridine and acetic anhydride (4:1, v/v) for 15min at 100°C. The product was again identified by comparison of its UV spectra (Fig.III-1.2) and EI/MS spectra (Fig. III-1.3) with those of CLN-Ac as described in Section II-1.

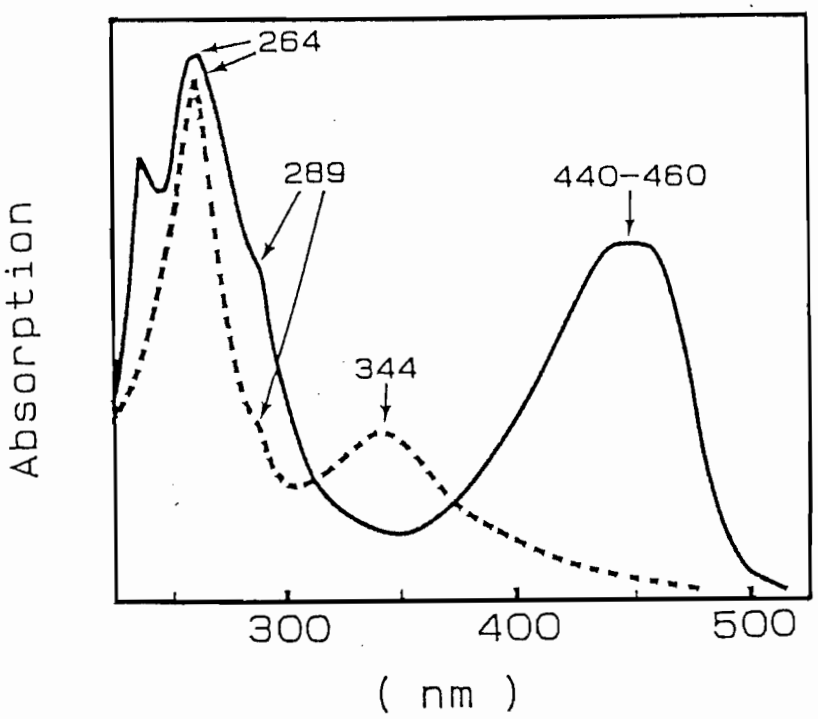


Fig. III-1.2. UV-VIS absorption spectra of CLN isolated from Makino soil and its tetraacetate in chloroform (———, CLN; ----, CLN-Ac).

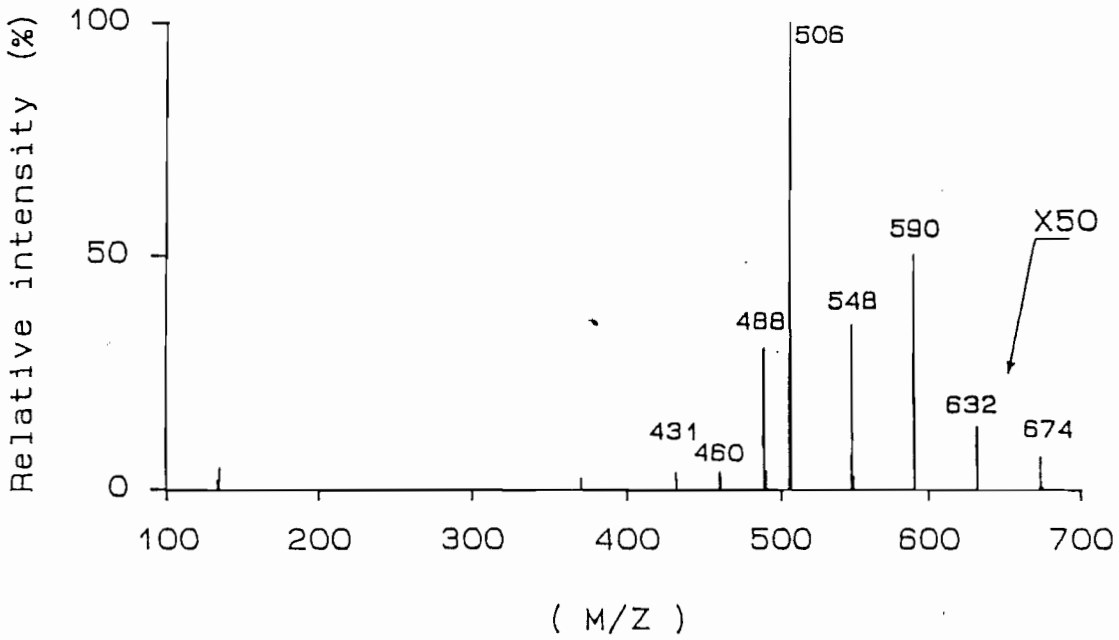


Fig. III-1.3. EI/MS spectra of CLN-Ac.

Sample preparation for HPLC analysis

The method of sample preparation is shown schematically in Fig. III-1.4. A soil sample (usually 2g) was shaken with a mixed solvent of acetone-2N **NaOH** (1:1, v/v; 4 ml) in a 50ml flask for 20sec. After the acetone layer as described by McGrath [1972] was obtained, the residual soil was shaken repeatedly with 4ml of acetone until no more color could be extracted from soil. Total acetone extracts were acidified and re-extracted with **$CHCl_3$** in a 100ml separatory funnel.

The **$CHCl_3$** extract was evaporated to dryness. The resulting residue was acetylated with pyridine and acetic anhydride (4:1, v/v; 0.25ml) for 15min at 100°C. The solvents were removed under vacuum and the acetylated product was dissolved in **$CHCl_3$** (0.5-1ml). The solution was applied to a mini column of dry-silica (2g) and the column was left overnight at room temperature to remove the solvent. Non-polar impurities were removed from the column by elution with 30ml of benzene. Subsequently, the residues on the column were eluted with 30ml of a mixture of **$CHCl_3$** and **EtOAc** (9:1, v/v). The eluate was evaporated to dryness and next dissolved in **$CHCl_3$** (0.5ml). An aliquot of the solution was used for **HPLC**.

Determination by HPLC analysis

HPLC was carried out with a Hitachi 655 **HPLC**: Sumipack column Partisil 5 ODS-2 (4.6X150mm I.D.); temperature, 22-25°C; mobile phase, methanol-water (80:20, v/v); flow rate, 0.8 ml/min; monitoring wave-length, 260nm.

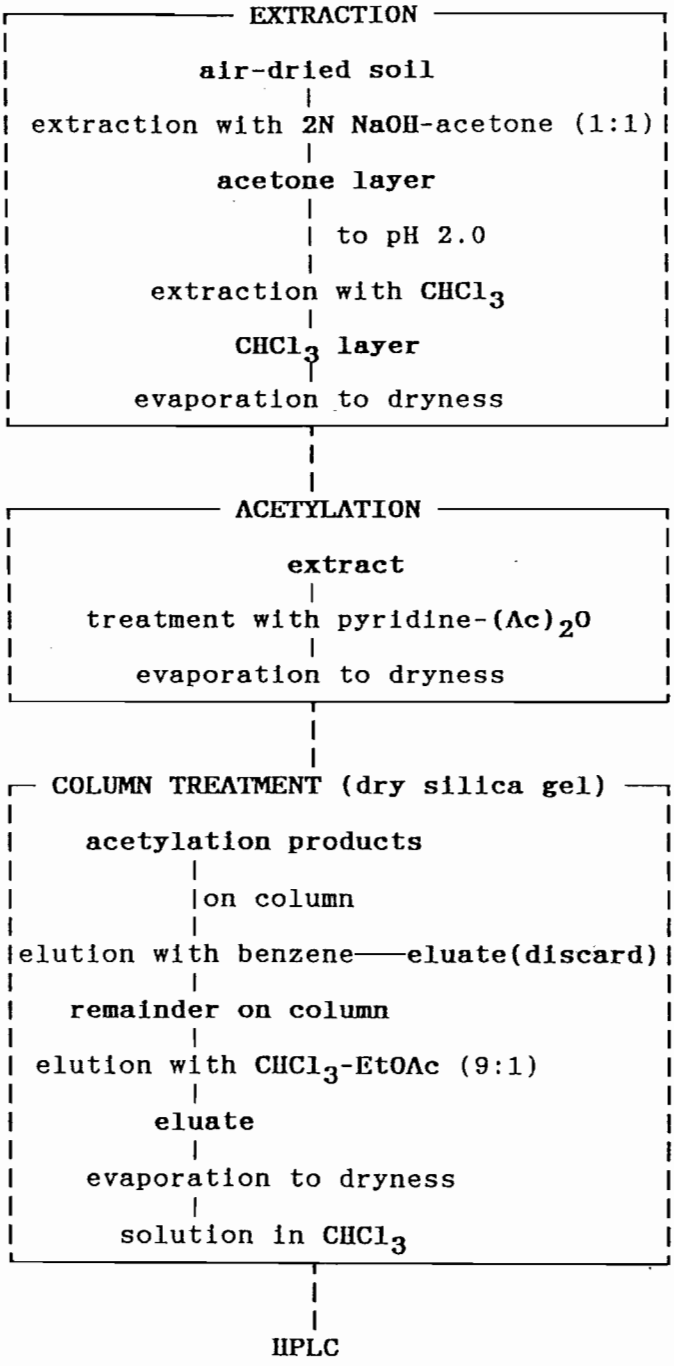


Fig. III-1.4. Sample preparation for HPLC analysis.

To obtain the calibration curve, various amounts of the standard **CLN** were examined upon acetylation and subsequent treatment. The peak on the chromatogram of each soil sample, which agreed with that of the standard sample in the retention time, was additionally analyzed by **EI/MS**. Both standard and soil samples were run in triplicate.

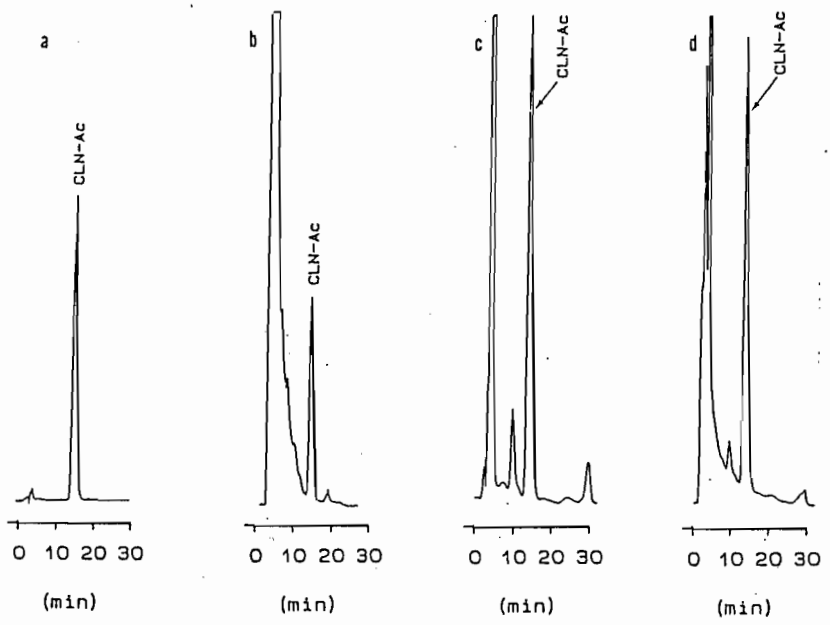
Colorimetric determination by McGrath's method

For all soil samples, the colorimetric determination of **CLN** was carried out using the method as reported by McGrath [1972].

RESULTS

Detection and separation of CLN-Ac by HPLC analysis

From the **CLN-Ac** standard, one peak was obtained with a retention time of 14.48min as shown in Fig. III-1.5a. This peak of the same retention time was observed in all samples from soils of different types with various carbon contents and additionally identified as **CLN-Ac** by **EI/MS**. Figs. III-1.5b-d represent the chromatograms containing a number of the other peaks. The peak shown in chromatogram (Fig. III-1.5b) corresponds to the minimum amount of **CLN** in all soil samples analyzed in this experiments. The chromatograms of all samples showed the satisfactory separation of **CLN-Ac** from other com-



Figs. III-1.5(a-d). HPLC chromatograms of (a) acetylated standard sample; (b) Azumi soil sample; (c) Isarigami soil sample; and (d) Hachibuse soil sample.

Table III-1.2. Contents of chrysotalunin in soils by HPLC and colorimetry.

Plot No.	Content of CLN			H/C** ratio
	by HPLC		by colorimetry (ppm)	
	(ppm)	% of T-C*		
HY-1	0.5	0.0019	0.0	-
HY-2	3.8	0.0109	6.2	61
HY-3	1.7	0.0024	3.5	49
HY-4	7.9	0.0184	12.2	65
HY-5	2.8	0.0078	6.6	42
HY-6	7.0	0.0090	8.6	81
HY-7	1.1	0.0009	4.5	24
HY-10	6.9	0.0329	8.5	81
HY-11	2.0	0.0037	2.1	95
HY-21	2.3	0.0026	6.3	37
HY-12	8.4	0.0077	13.7	61
HY-13	12.4	0.0170	13.1	95
HY-14	25.8	0.0211	26.0	99
HY-15	7.0	0.0045	8.6	47
HY-16	27.7	0.0157	29.3	95
HY-17	8.5	0.0096	13.1	65
HY-18	9.2	0.0051	14.0	66

* T-C is total organic carbon and was determined by rapid dichromate oxidation method [Page et al, 1982].

** H/C ratio = content of CLN by HPLC / content of CLN by colorimetry X 100

Table III-1.2. Contents of chrysotalunin in soils by HPLC and colorimetry.

Plot No.	Content of CLN			H/C** ratio
	by HPLC		by colorimetry (ppm)	
	(ppm)	% of T-C*		
HY-1	0.5	0.0019	0.0	-
HY-2	3.8	0.0109	6.2	61
HY-3	1.7	0.0024	3.5	49
HY-4	7.9	0.0184	12.2	65
HY-5	2.8	0.0078	6.6	42
HY-6	7.0	0.0090	8.6	81
HY-7	1.1	0.0009	4.5	24
HY-10	6.9	0.0329	8.5	81
HY-11	2.0	0.0037	2.1	95
HY-21	2.3	0.0026	6.3	37
HY-12	8.4	0.0077	13.7	61
HY-13	12.4	0.0170	13.1	95
HY-14	25.8	0.0211	26.0	99
HY-15	7.0	0.0045	8.6	47
HY-16	27.7	0.0157	29.3	95
HY-17	8.5	0.0096	13.1	65
HY-18	9.2	0.0051	14.0	66

* T-C is total organic carbon and was determined by rapid dichromate oxidation method [Page et al, 1982].

** H/C ratio = content of CLN by HPLC / content of CLN by colorimetry X 100

contained 0.5ppm ; 8 samples of brown forest soils (Dystric or Humic Cambisols) ranged from 1.1 to 7.9ppm ; 8 samples of black soils (Humic Andosols) ranged from 7.0 to 27.7ppm.

Comparison of HPLC and colorimetric determination

The CLN contents of each soil were assayed using the colorimetric method [McGrath, 1972] to compare its accuracy with results obtained by the HPLC method. The H/C ratios (Table III-1.2) represent the ratio of the amounts estimated by HPLC

to those by the colorimetric method. The H/C ratios ranged from 24 to 99.

As shown in Table III-1.2, the results obtained by the colorimetry are not very different from those of McGrath's report [McGrath,1972]. However, although the presence of CLN in red soils could not be detected by the colorimetric method, small content (0.5ppm) of that could be determined by the HPLC method.

DISCUSSION

There are several difficulties concerning the isolation of CLN from soils and its characterization and quantification: these include the need for special solvents (alkaline acetone and alcohols, concentrated sulphuric acid, and hot $CHCl_3$); its extremely low solubility; its strong adsorption on the surface of silica gel and glassware.

McGrath [1972] determined CLN using simple colorimetry. However, his method often requires greater amounts of soil in case of the determination of trace amounts. Furthermore, this determination is sometimes only approximate because of the interference with other organic compounds in soils. The low values of H/C ratios such as 24 and 37 in soils of HY-7 and HY-21, respectively, in our present data, suggest that the

colorimetric determination is very susceptible to interference from impurities.

In these experiments, we showed that the HPLC method is sufficiently rapid, reliable and sensitive for the determination of CLN in soils of different types with various carbon contents.

According to McGrath [1967, 1972], Matsui and Kumada [1974] as well as Foo and Tate [1977], CLN was detected in Irish, Canadian, Japanese and New Zealand soils. This experiment also demonstrated that its amounts in Japanese soils was not very different from those in soils of these other countries [McGrath, 1972; Foo and Tate, 1977]. These findings suggest its comparable world-wide distribution in concentrations.

Although the numbers of samples were not enough to discuss for the relation between amounts of CLN and soil types, it seems that the CLN contents of black soils are higher than those of brown forest soils and a red soils. Since black soils have a thick black-colored A horizon which is frequently very high in humus (total carbon) content [Forest Soil Division, 1976], it is liable to be presumed that their higher CLN contents depend on their total carbon contents. However, no obvious relationship between CLN contents and total carbon contents was apparent, as shown in Table III-1.2. Generally, it seems that the higher humus accumulation is regarded as a great deal of active alumina produced as a weathering product of volcanic ash -- parent materials of the black soil. McGrath [1972] and Foo and Tate [1977] reported that CLN was

greatly concentrated in the Bh horizons of Podozols, while it is well-known that hydroxyanthraquinones have strong chelate effects [Qureshi et al., 1979]. Therefore, the distribution of **CLN** may be related to the existence of a particular form of certain minerals, such as aluminum and iron hydroxide polymorphs.

III-2. Distribution of Chrysotalunin in Various Surface Soils of Japan

MATERIALS AND METHODS

Soil samples

Sixty seven samples of surface soil were taken from wide location of Japan (Fig. III-2.1). The soil samples of sixty two were chosen from the surface horizons (A_{11} or A horizons) under grasses or forests. Five samples of surface soil were taken from paddy fields. Some properties of these soil samples were shown in Table III-2.1. The air-dried samples were ground to pass a 0.25mm screen for the following analysis.

Determination of CLN in soils

The determination of CLN in soils was carried out using HPLC procedure following the acetylation of CLN. Detailed procedures were described in Section III-1. The weight of samples for determination were chosen in order to contain 100mg equivalent weight of carbon.

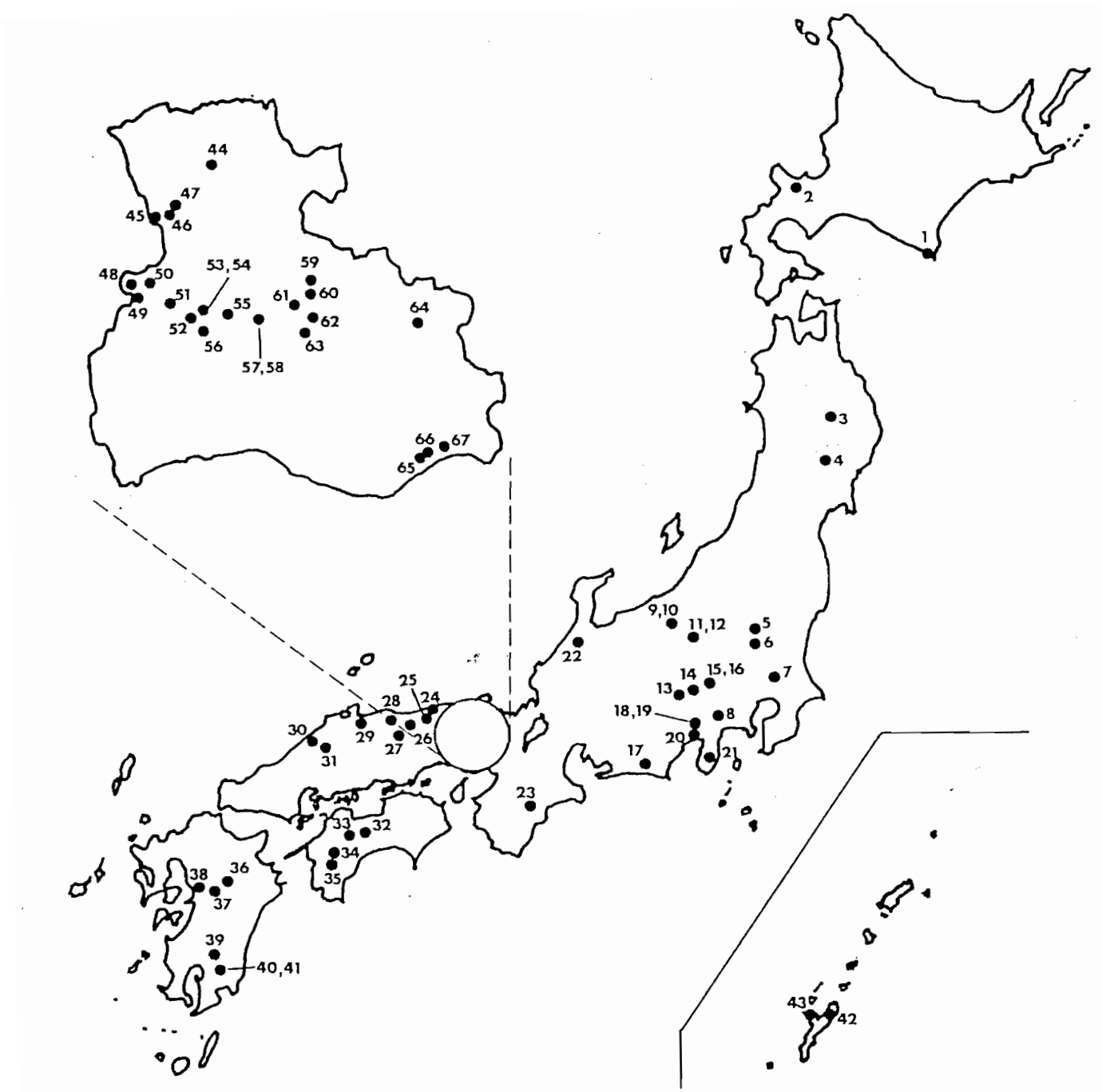


Fig. III-2.1. Location of sampling sites.

Table III-2.1. Soil samples used.

Soil Nos.	Sample Name	Depth (cm)	Soil* Types	Types of** Land Use	Remarks***
1	Erimo	0-19	Bl	G	Pasture
2	Niseko	0-15	Bl	G	Bamboo grass
3	Iwate Univ.	0-23	Bl	G	Pasture
4	Kanagasaki	0-11	Bl	F	Red pine, Bamboo grass
5	Kanuma	0-23	Bl	-	Planted rhododendron
6	Imaichi	0-13	Bl	F	Broad-leaved forest
7	Yatabe	0-12	Bl	G	Wild grass land
8	Fuji-sunaga	0-30	Im	F	Wild land
9	Nojiriko-1	0-18	Bl	F	Cedar, Oak
10	Nojiriko-2	0-17	-	P	Paddy field
11	Sugadaira	0-23	Bl	G	Eulalia
12	Nekodake	0-17	Bl	G	Bamboo grass
13	Yatsugadake	0-12	Bl	F	Oak
14	Jumonji	0-15	P_D	F	Beech
15	Akazawa-1	0-22	B	F	Cypress
16	Akazawa-2	0-11	B	F	Cedar
17	Iwata	0-9	Bl	F	Red pine, Cypress, Cedar
18	Fujinomiya-1	0-8	B	F	Cypress, Beech
19	Fujinomiya-2	0-15	Bl	F	Cypress, Eulalia
20	Fujinomiya-3	0-15	Bl	F	Deciduous broad-leaved forest
21	Yugashima	0-10	lBl	F	Ceder, Oak
22	Hakusan	0-7	P_D	F	Beech
23	Soni	0-15	Bl	G	Bamboo grass Eulalia
24	Sakyuken	0-20	Im	F	Red pine
25	Tottori Univ.	0-18	Bl	F	Poplar, Bamboo grass
26	Sekigane	0-13	Bl	F	Ceder
27	Hirusen	0-7	Bl	F	Oak
28	Daiei	0-18	Bl	F	Cypress, Red pine, Bamboo grass
29	Yonago	0-10	B	F	Chinquapin, Oak
30	Sanbe	0-18	Bl	F	Red pine, Bamboo grass
31	Akagi	0-17	Bl	F	Ceder, Bamboo grass
32	Kanpuzan	0-8	Bl	F	Beech, Bamboo grass
33	Yosakoi	0-5	B	G	Bamboo grass
34	Mikawa	0-6	Bl	G	Bamboo grass
35	Godan	0-8	Bl	G	Bamboo grass, Eulalia
36	Aso	0-15	-	P	Paddy field
37	Otsu	0-22	Bl	G	Alang grass
38	Nishigoshi	0-30	Bl	G	Alang grass
39	Kobayashi	0-20	-	P	Paddy field
40	Miyakonojo-1	0-18	-	P	Paddy field
41	Miyakonojo-2	0-13	Bl	G	Pasture
42	Nishimeidake	0-8	Y	F	Chinquapin
43	Motobu	0-8	R	G	Wild grass land
44	Kannabe	0-21	Bl	F	Red pine, Bamboo grass
45	Hachiura	0-20	B	F	Ceder, Bamboo grass
46	Hachibuse-1	0-8	Bl	G	Bamboo grass, Eulalia
47	Hachikita	0-20	Bl	F	Cypress, Beech

Table III-2.1. Cont'd.

Soil Nos.	Sample Name	Depth (cm)	Soil* Type	Type of ** Land Use	Remarks***
48	Chigusa-1	0-29	B	F	Ceder
49	Chigusa-2	0-28	B	F	Ceder
50	Mimuro	0-21	Bl	F	Cypress, Bamboo grass
51	Saiki	0-22	B	F	Cypress, Bamboo grass
52	Azumi	0-18	R	F	Ceder
53	Yamada	0-20	dB	F	Cypress
54	Nakatsubo	0-10	B	F	Cypress
55	Mineyama	0-22	Bl	F	Ceder, Bamboo grass
56	Sugyome	0-10	Bl	F	Cypress, Bamboo grass
57	Oyama-1	0-5	dB	F	Cypress
58	Oyama-2	0-8	B	F	Cypress
59	Mitani	0-17	B	F	Cypress
60	Todoroki	0-22	B	F	Cypress
61	Isarigami	0-25	B	F	Cypress, Eulalia
62	Makino	0-24	Bl	G	Bamboo grass, Eulalia
63	Yamanobe	0-5	B	F	Cypress
64	Mima	0-20	-	P	Paddy field
65	Maya	0-34	dB	F	Chinquapin
66	Nagamine	0-7	B	G	Bamboo grass
67	Kinchozan	0-18	B	F	Chinquapin

* Type of soils are determined on the basis of the classification of forest soil in Japan by Forest Soils Division of Japan [1976], except for soils of paddy soils, as follows: P_D, dry podzolic soils; B, brown forest soils; dB, dark brown forest soils; Bl, black soils; lBl, light colored black soils; R, red soils; Y, yellow soils; Im, immature soils.

** Type of land use are as follows: P, paddy field; F, forest; G, grass land.

*** Bamboo grass, Sasa and/or Neosasamorpha sp.; Eulalia, Miscanthus Sinensis; Red pine, Pinus densiflora; Ceder, Cryptomeria japonica; Oak, Quercus sp. ; Beech, Faguceae sp.; Chinquapin, Castanopsis sp.; Cypress, Chamaecyparis obtusa; Alang grass, Imperata cylindrica.

RESULTS AND DISCUSSION

General distribution of CLN in Japan

In the previous section, we established a new method for isolative determination of chrysotalunin (**CLN**) by high-performance liquid chromatography. In this experiments, **CLN** was examined in 67 soil samples from the wide location in Japan as shown in Fig. III-2.1. The contents of **CLN** were listed in Table III-2.2. All soil samples gave its content ranging from 0.1 to 29.1ppm. The maximum content was found in Godan soil (No.35), black soils of Kochi Prefecture and the minimum content was in Sakyuken soil (No.24), immature soils of Tottori Prefecture. These soil samples consisted of black soils, brown forest soils, red soils, yellow soils, podzolic soils and even immature soils, and widely located from the southern subtropical region to the northern subarctic region in Japan. The results suggests that **CLN** is universally present in Japanese soils.

According to McGrath [1972], 25 soils in Ireland including acid brown earths, brown podzolic soils and gray podzolic soils, and one podzolic soils in Canada gave a positive reaction for **CLN** or very similar compound. Other investigators had found **CLN** in soils of brown forest soils, ando soils, alpine meadow soils and high mountain grass land soils in Japan [Matsui and Kumada, 1974], and of kauri podzolic soils in New Zealand [Foo and Tate, 1977]. These reports suggested its

Table III-2.2. Some general properties and CLN content of soil sample.

Soil Nos.	pH (H_2O)	T-C (%)	DCB-Fe (%)	TM-Al (%)	CLN (ppm)
1*	5.5	15.9	2.15	2.48	2.8
2*	5.6	11.3	1.28	0.58	8.3
3*	5.4	8.8	3.73	9.49	1.2
4*	5.0	14.5	4.28	5.95	6.0
5*	5.3	7.2	4.36	9.63	2.5
6*	5.2	19.8	3.11	8.91	15.2
7*	5.4	7.9	6.23	8.53	7.4
8	6.1	ND	ND	ND	0.3
9	4.6	13.1	3.00	4.98	5.0
10	5.6	13.3	5.77	4.19	2.3
11	5.1	17.3	3.94	6.13	11.1
12	3.9	21.5	2.13	1.72	4.1
13	4.7	11.8	5.21	3.97	1.9
14	4.0	7.3	0.92	0.88	0.3
15	4.1	12.0	2.09	2.78	0.4
16	4.8	19.8	2.19	4.35	2.1
17*	5.0	6.5	1.90	1.87	5.2
18*	5.9	19.6	4.79	8.54	1.8
19*	5.6	26.3	4.36	8.81	4.0
20*	5.4	18.9	6.89	11.59	5.5
21*	4.6	20.6	1.34	2.11	11.2
22	4.9	2.9	ND	ND	0.6
23	5.2	13.2	1.31	1.07	22.8
24	5.1	0.1	ND	ND	0.1
25	5.0	8.3	1.11	3.97	4.3
26	4.3	20.1	1.67	2.21	9.4
27	5.5	11.8	1.40	4.00	16.6
28	5.0	14.8	1.21	4.41	7.1
29	5.0	5.1	1.29	0.99	2.7
30	5.1	7.7	0.33	2.72	11.8
31	5.1	12.9	0.81	4.42	19.0
32	4.7	15.0	2.59	0.94	25.0
33	4.6	4.9	1.90	0.43	19.0
34	4.5	17.9	1.87	1.04	24.6
35	4.6	17.4	1.48	0.96	29.1
36*	6.1	4.8	2.77	3.84	0.4
37*	5.7	3.7	7.44	13.11	1.1
38*	6.0	9.9	5.54	9.98	1.2
39*	5.9	6.8	1.96	6.39	3.0
40*	5.8	6.1	0.82	4.66	2.5
41*	6.3	8.0	2.09	6.80	3.5
42	5.5	15.0	1.44	0.33	2.2
43	4.2	3.8	1.47	0.16	0.9
44	5.5	8.9	3.91	3.93	9.2
45	5.3	2.1	1.86	1.27	6.9
46	5.7	21.0	2.26	1.42	27.7
47	5.5	15.4	1.40	1.71	7.0

Table III-2.2. Cont'd.

Soil Nos.	pH (H_2O)	T-C (%)	DCB-Fe (%)	TM-Al (%)	CLN (ppm)
48	4.8	4.4	1.81	1.31	2.2
49	4.9	6.1	1.71	1.02	2.8
50	5.1	22.1	1.96	1.56	8.5
51	5.2	8.5	1.76	0.50	6.9
52	4.4	2.7	1.01	0.19	0.5
53	4.9	3.5	2.51	0.68	3.8
54	5.0	8.5	1.12	0.45	0.7
55	5.0	10.9	2.73	0.68	8.4
56	4.4	7.3	2.60	1.36	12.4
57	4.7	12.6	2.79	0.53	1.1
58	4.3	8.9	1.80	0.90	2.3
59	4.4	7.8	1.18	0.80	7.0
60	4.1	10.8	2.00	0.64	2.8
61	3.6	4.3	2.04	0.59	7.9
62	4.6	12.2	1.69	1.78	25.8
63	4.1	7.2	1.16	1.56	1.7
64	6.1	9.3	1.81	1.99	0.8
65	4.1	5.4	0.76	0.30	2.0
66	4.3	9.1	1.41	0.59	18.0
67	4.4	3.1	0.78	0.19	0.2

T-C, total carbon; DCB-Fe, dithionite-citrate extractable Fe; TM-Al, oxalate-oxalic acid extractable Al. ND, not determined.

* analytical data except for CLN content are cited from the book of Ando soils in Japan [Wada, 1986].

world-wide distribution and its presence in soils of various types. However, there were also a number of negative soils for CLN in their reports. The fact that all soil samples gave positive data for CLN in our experiments, is likely due to the difference of its detectable level by each method. Therefore, the distribution of CLN may be more universally in soils of the world.

Distribution patterns of CLN among soil types

In Section III-1, although the numbers of samples were not enough to discuss for the relation between amounts of CLN and soil types, it seems that the CLN contents of black soils are higher than those of brown forest soils and red soils.

In this experiments, the mean values of CLN contents in the soils of different types were shown in Table III-2.3. The value of black soils was 10.2 (±1.4, SE) ppm (N=36), and was about two times as high as that of brown forest soils (4.8±1.2 ppm, N=19). Although the number of samples was not enough, soils of other types such as yellow soils, red soils and podzolic soils gave lower contents of CLN than black soils and brown forest soils. The frequency distributions of the origi-

Table III-2.3. Mean values of CLN contents in the soils of different types (mean±SE).

Soil Types	Numbers of Sample	CLN* (ppm)	Log CLN
Black soils	36	10.2±1.4	0.85±0.07
Brown forest soils	19	4.8±0.1	0.43±0.11
Soils of other types**	7	0.7±0.1	-0.32±0.15
Total***	62		

* The values are significantly difference among three kind of soil types ($p<0.05$).

** Soils of other types consist of yellow, red, dry podzolic and immature soils.

*** Except for paddy soils.

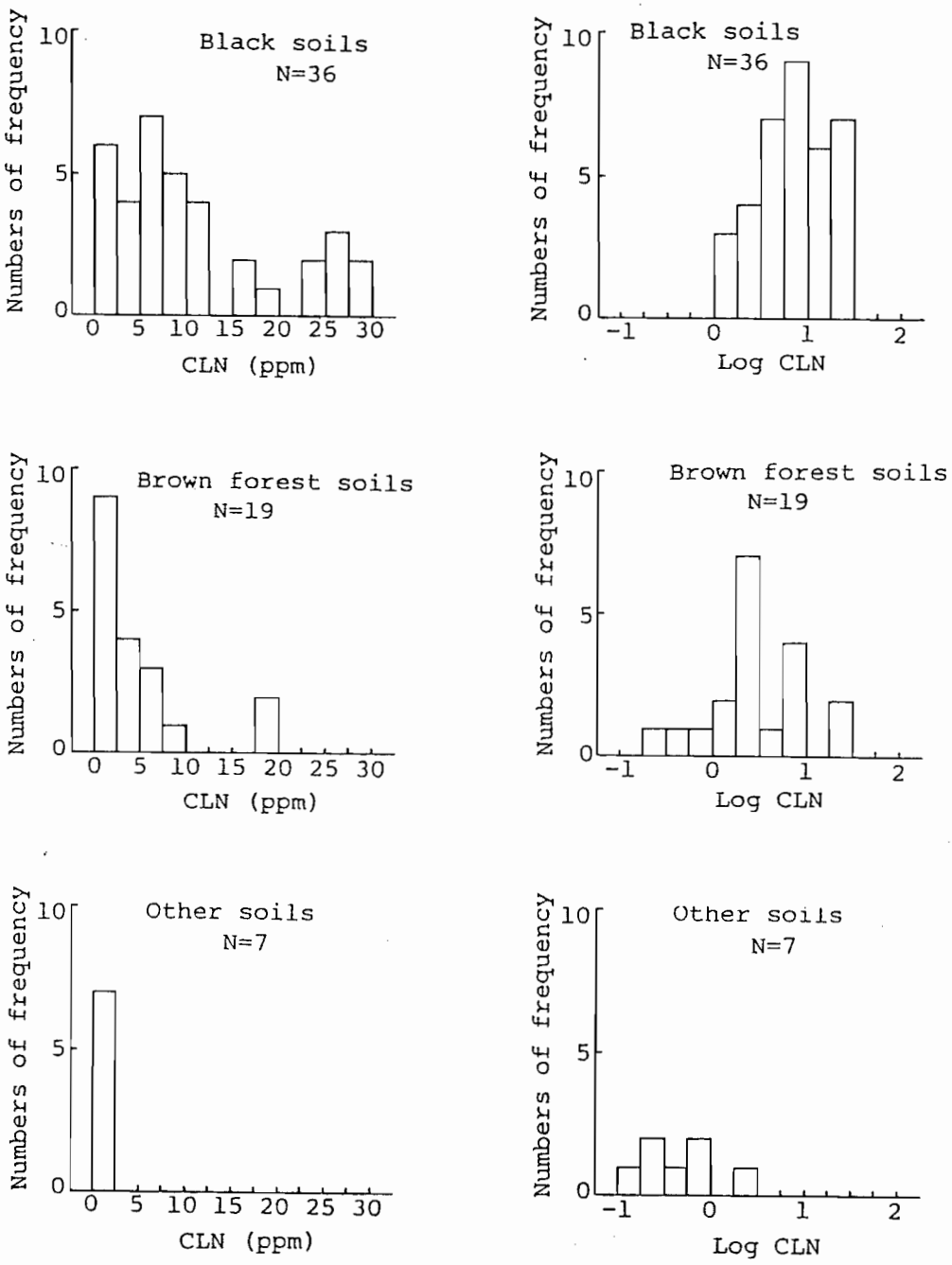


Fig. III-2.2. Frequency distributions of CLN contents in different soil types.

nal and the log-transformed **CLN** contents in each soil of different types are illustrated in Fig. III-2.2. It is clear from the histogram of the log-transformed data that distribution patterns are different among the three groups of soil types. Significant difference ($p<0.05$) was found among them according to the U test.

Generally, black soils have higher organic matter content than the soils of other types. However, relation between **CLN** content and the total carbon content was no apparent as shown in Table III-2.2. The relation of **CLN** content with the other general chemical data could not be also pointed out. As another properties of the black soils, it is well-known that these soils containe highly the amorphous minerals (e.g. allophane, allophane-like constituents, iron or aluminum hydroxide polymorphs, etc.) derived from volcanic ash. Thus, the high accumulation of **CLN** in the black soils may depend on the its close association with a particular form of certain mineral in these soils.

Distribution patterns of CLN in soils under different land use

The mean values of **CLN** contents in soils under different land use were shown in Table III-2.4. At the first, all soil samples were divided to three groups such as paddy fields, grass lands and forests. However, in the soil samples of the grass lands, the **CLN** contents in the glass land soils under bamboo grass (*Sasa sp.* or *Neosasamorpha sp.*) and/or eulalia

Table III-2.4. Mean values of **CLN** contents in the soils under different land use (mean±SE).

Land use	Numbers of Sample	**CLN*** (ppm)	Log **CLN**
Paddy fields	5	1.8±0.1[a]	0.15±0.15
without bamboo grass and/or Eulalia			
Grass lands (cont. pasture)	8	2.3±0.8[a]	0.19±0.14
Forests	28	3.7±0.1[a]	0.30±0.10
with bamboo grass and/or Eulalia			
Grass lands	10	19.0±2.6[b]	1.22±0.08
Forests	15	9.9±1.4[c]	0.94±0.05
Total**	66		

* The values are significantly different among soils under land use with different superscripts ($p<0.05$).

** Except for Kanuma soil (No. 5)

(*Miscanthus sinensis*) (**BE**) were clear different from those in the soils under the other grasses. Therefore, all samples were re-devided to five groups as follows; paddy fields (**PF**), grass lands under **BE** (**BEG**) and without **BE** (**nBEG**), forest with **BE** (**BEF**) and without **BE** (**nBEF**). The frequency distributions of the log-transformed **CLN** contents in each soil groups were also showed in Fig. III-2.3. It is clear that their distribution patterns are different among **BEG**, **BEF** and the other soil groups. Significant difference ($p<0.05$) was found among **BEG**, **BEF** and the other soil groups. The significant difference

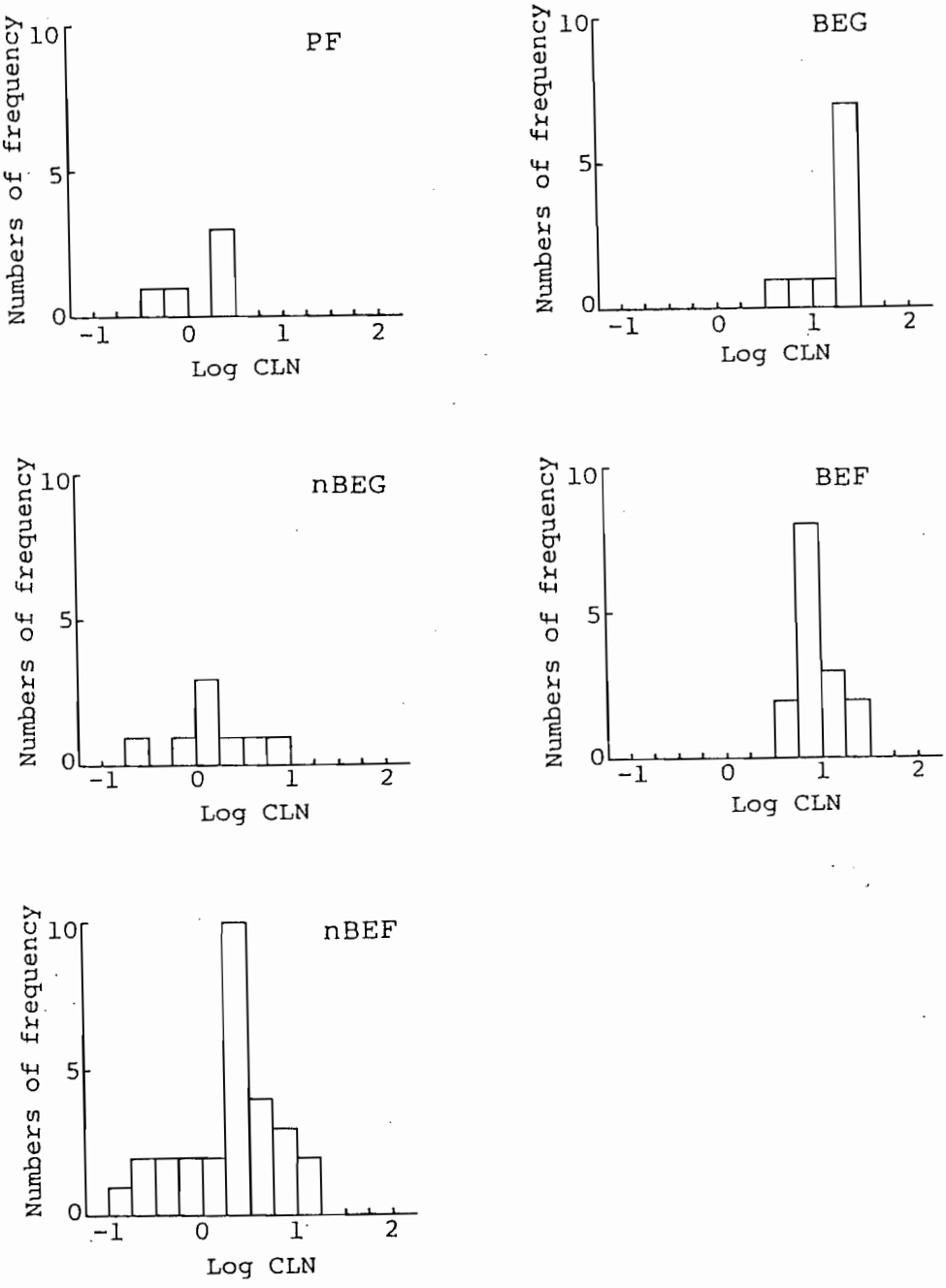


Fig. III-2.3. Frequency distributions of Log-transformed CLN contents in different groups of land use

among PF, nBEF and nBEG could not be pointed out as the number of the samples was not enough.

The maximum of the mean value was 19.0±2.6ppm in BEG (Table III-2.4). As shown in Fig. III-2.3, CLN contents in most soil of BEG distributed in such very high values as the range from 1.25 to 1.50 (Log CLN content).

McGrath [1972] presumed that CLN was of microbial origin, because soil fibers and overlying vegetations (heath and grass) existed at the site sampling soil containing very high value of CLN did not contain CLN and the other anthraquinones. It is therefore, probably that the high accumulation in soils of BEG is resulted not from the presence of bamboo grass and/or eulalia but from the presence of microorganisms to connect with such vegetations.

In this experiments, of particular interest is the high accumulation of CLN in the black soils and in the soils under bamboo grass and/or eulalia. Since the black soils are distributed mainly on grasslands, it is now considered that grassland vegetation is a very important factor in the formation of the black soils. However, two brown forest soils under BEG (Soil Nos.33 and 66) gave the high CLN contents and in two black soils under algang grass (Soil Nos.37 and 38) gave its low contents. Therefore, it is probably considered that the high accumulation of CLN in the black soils are mainly influenced by interaction between CLN and minerals in these soils,

while those in the soils of **BEG** are mainly influenced by the productivity of **CLN** from microorganisms to connect with such vegetations.

III-3. Distribution Pattern of Chrysotalunin in Soil Profiles

MATERIALS AND METHODS

Soil samples

1) Profiles of four black soils, one brown forest soils, one red soils, and one podzolic soils were chosen from sampling plots of the soil examined in Section III-2. The surface soils of these profiles had the higher contents of CLN in each soil types. Soil samples were taken from each horizon of these profiles. Photographs of each profile were shown in Fig. III-3.1.

2) Soil samples were taken from the surface and the buried humus horizons of Subashiri soil profile in Shizuoka Prefecture. This profile held several key horizons with known radio carbon ages [Uesugi and Yonezawa, 1987]. Two samples of surface soils were taken from the surface (0-10cm) and Hoei scoria (10-60cm, <1,700 year before past (YBP)). Tree samples of buried humus soils were taken from Fuji-Kurotsuchi horizons (17.4-20.2m, 6,400-10,200 YBP).

3) Soil Samples were taken from each A horizon (A_{11}, A_{12} and A_3) at Hachibuse-II soil profile, in Hyogo Prefecture. Location of Hachibuse-II is near by that of Hachibuse-I. Both soil profiles were same type of soil.

These soil samples were air-dried and passed through a 2mm

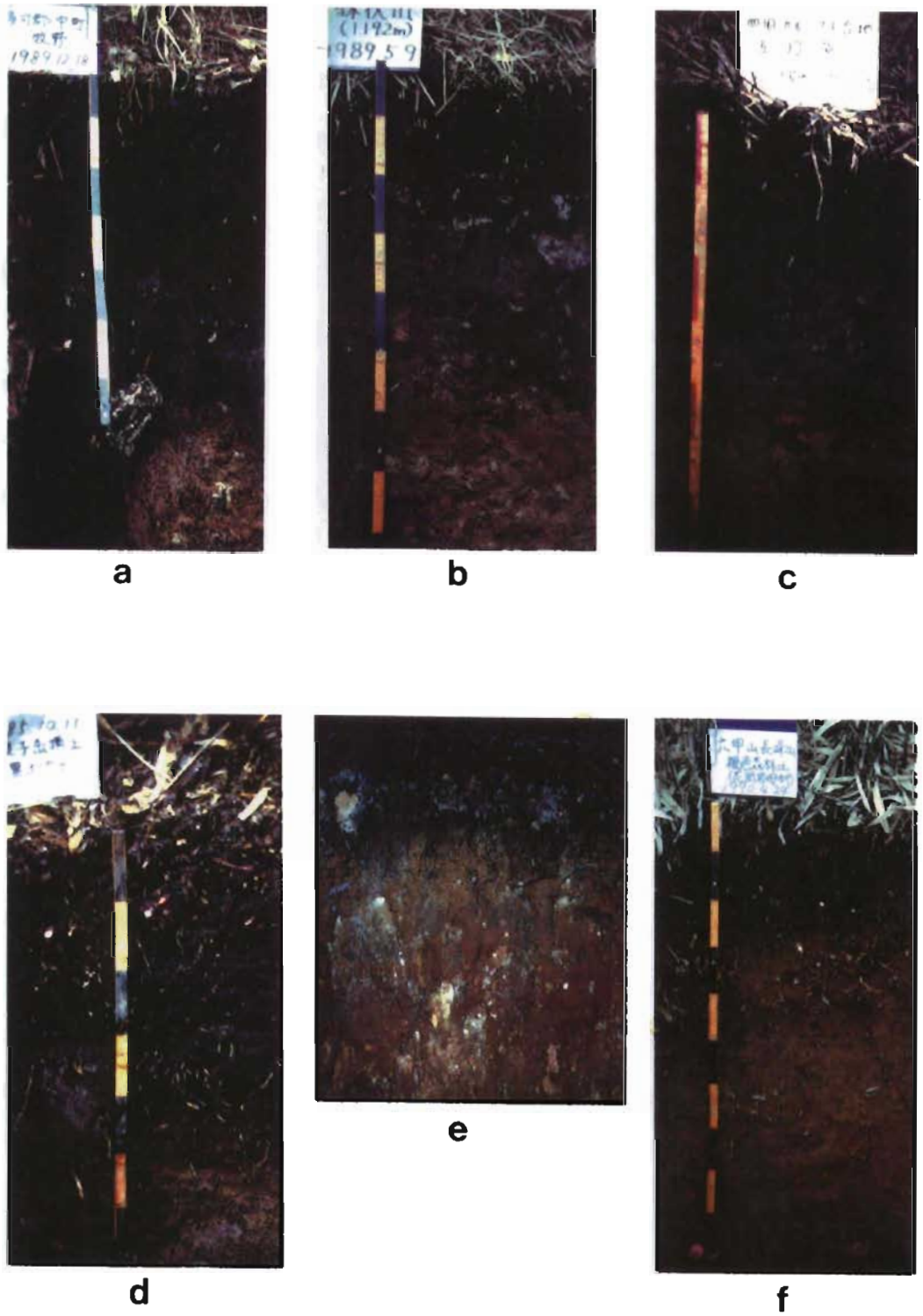


Fig. III-3.1. Photographs of sampling soil profiles; (a) Hachibuse-I, (b) Makino, (c) Godan, (d) Nekodake, (e) Motobu, (f) Nagamine.

sieves. A portion of the soil sample was ground to pass a 0.5mm sieves for the following analysis.

General analysis

Soil pH was measured by the grass electrode method (soil-water ratio 1:2.5). Dithionite-citrate extractable (**DCB**) and oxalate-oxalic acid extractable (**Tamm**) solutions were obtained as described in the book of Ando soil in Japan [Wada, 1986]. The contents of iron and aluminum in each solution were determined by atomic absorption analysis. Total carbon content was measured by rapid dichromate oxidation method [Page et.al.,1982].

Determination of CLN

The determination of **CLN** in soils and humus materials was carried out by the **HPLC** procedure coupled with the acetylation of **CLN**. Detailed procedures were described in Section III-1. Soil samples were used of 100mg equivalent weight of carbon. Humus materials were also used of that of carbon in the unfractionated soil.

Determination of BP

A soil sample (20-50g) was shaken with a mixed solvent of acetone-2N **NaOH** (1:1, v/v) as described by McGrath [1972]. Total acetone extracts were acidified and extracted with **EtOAc**.

The EtOAc extract was shaken with enough 5% NaOH. The NaOH extract was acidified and extracted with EtOAc. The EtOAc extract was evaporated and the residue was chromatographed on silica gel by eluting with $CHCl_3$. The eluate was evaporated and the residue was dissolved in $CHCl_3$. A portion of the solution was applied for HPLC. The conditions for HPLC were as follows; the column was Partisil 5 ODS-2 (4.6X150 mm), the mobile phase was 90% ethanol in water (flow rate, 0.8ml/min.), the monitoring wave-length was 286nm. To obtain the calibration curve, the solutions of various concentration of the pure BP (see in Section II-1) were used; their concentration were estimated on the basis of the data of its molar extinction coefficient [Gluchoff et al., 1972]. The chromatograms of pure BP and samples of A_{11} horizons in Makino soil were shown in Fig. III-3.2.

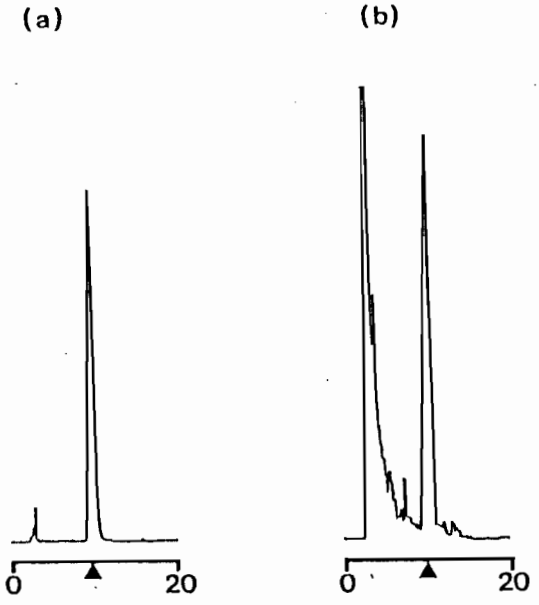


Fig. III-3.2. HPLC chromatograms of (a) the pure pigment (BP) and (b) Makino soil (A_{11} horizon).

Preparation of humus materials

The method for preparation of humic acid (HA), fulvic acid (FA) and humin (HN) fractions used for determination of CLN was as follows. Soil samples of each A horizon in Hachibuse-II soil profile were used.

To an air-dried soil sample (<0.5mm, containing 100mg of carbon) in 200ml centrifuge tube was added 60ml of 0.1N NaOH, followed by heating at 100°C for 30min. After cooling in an ice-water bath, the tube was centrifuged at 12,000rpm for 10min. After alkaline extract was decanted, the residue (HN fraction) washed with 20ml of distilled water was centrifuged as described before. The alkaline extract and the washing was combined, and then acidified to pH 2.0 with conc. H_2SO_4 (1ml/100ml). The extract was settled for one hour, and then centrifuged as described before. After the supernatant was decanted, the residue (HA fraction) washed with 20ml of 0.1N H_2SO_4 was centrifugated as described before. The FA fraction was obtained by combining the supernatant with the washing.

The humus composition was determined by the method of heating extraction with 0.1N NaOH as reported by Oba [1964]. The classification of humic acid (HA) types (A, B, P and Rp type) were determined from Δ log K and R_f values, which were calculated from the results of the humus composition analysis [Oba, 1964].

Determination of iron and aluminum content in successive extraction with NaOH, $Na_4P_2O_7$ and $Na_4P_2O_7$+$NaBH_4$

The extraction was based on the method described by Tsutsuki and Kuwastuka [1989]. Soil samples of each A horizon in Hachibuse-II profile were used.

A soil sample was successively extracted with 0.1N **NaOH** and 0.1M $Na_4P_2O_7$ in steam bath. Each extraction was repeated three times (30+30+20ml). To the residue, 100mg of $NaBH_4$ and 30ml of 0.1M $Na_4P_2O_7$ were added, left to stand overnight, centrifuged and washed two more times with $Na_4P_2O_7$. An aliquot of each extracted solution was acidified and dried. Then, the residue was heated at 750°C for 2 hours and was dissolved in conc. **HCl**. The solution was filled up with distilled water. Another portion of the solution extracted with **NaOH** was acidified to pH 2 and filtered (Toyo filter paper No.6). The filter paper with residue was heated and dissolved and then the solution was filled up with distilled water as described above.

Iron and aluminum were determined by atomic absorption analysis.

RESULTS AND DISCUSSION

Distribution patterns of CLN in soil profiles

Distribution of total carbon and **CLN** contents to the depth of the horizons in each soil profile are shown in Fig. III-3.3(a-f). Other chemical data of each soil sample were listed in Table III-3.1.

All soil profiles showed ordinary distribution of carbon in the figures.

Hachibuse-I soil profile showed a increasing content of **CLN** from 22 ppm at the surface horizon (A_{11}) to a maximum of 48 ppm at the A_{12} horizon following by the ordinary decrease with depth, as shown in Fig. III-3.3a. Makino soil profile also tended to show the same features (Fig. III-3.3b). Although in Godan and Nekodake soil profiles, the A_{13} and the A_3 horizons respectively gave the maximum contents of **CLN** (Figs. III-3.3c and d), the vertical distribution patterns of **CLN** contents in profiles of black soils had the maximum peak at the second or third horizons. The **CLN** contents of Motobu soil profile of red soils (Fig. III-3.3e) showed a slightly increasing to the second horizon (B_{21}) and a slightly decreasing to the next lower horizon (B_{22}) following by the rapid decrease with depth. In Nagamine soil profile, **CLN** contents in each horizon taperingly decreased from 19 to 0.5ppm with depth (Fig. III-3.3f). The characteristics of distribution patterns such as high accumulation of **CLN** in subsurface horizons is not apparent in

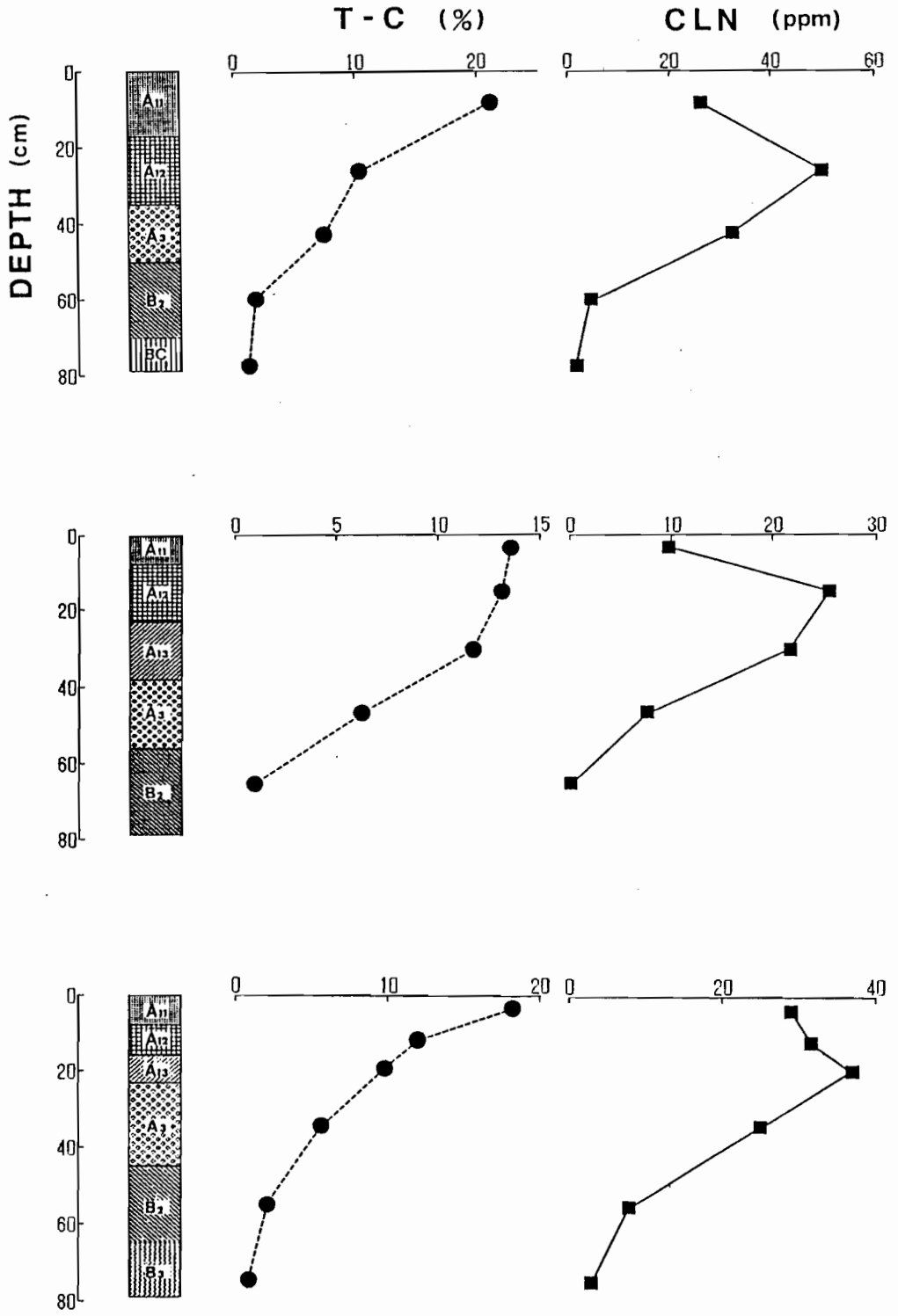


Fig. III-3.3(a-c). Distribution of carbon and CLN contents in soil profiles; (a) Hachibuse-I, (b) Makino, (c) Godan.

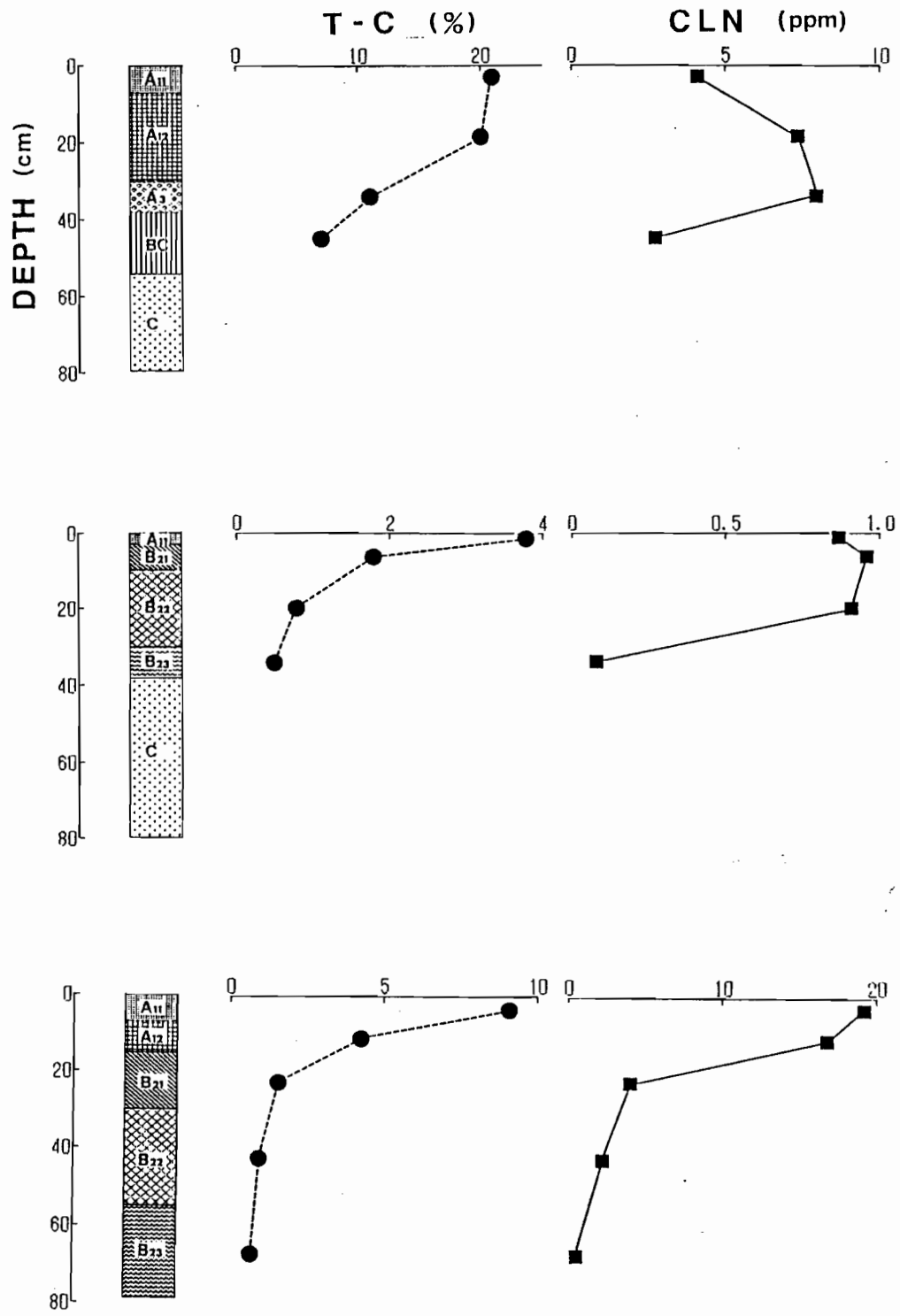


Fig. III-3.3(d-f). Distribution of carbon and CLN contents in soil profiles; (d) Nekodake, (e) Motobu, (f) Nagamine.

Table III-3.1. Chemical properties and CLN contents in soil profiles.

Soil Samples (depth, cm)	T-C (%)	pH (H2O)	CLN (ppm)	DCB ext.(%)		Tamm ext.(%)	
				Fe	Al	Fe	Al
Black soils							
Hachibuse-I (No.46)*							
A_{11} (0-17)	21.0	4.45	26.26	2.26	4.06	1.37	10.42
A_{12} (17-35)	10.4	4.90	49.80	2.52	7.57	1.76	30.25
A_3 (35-50)	7.3	4.85	32.39	2.49	0.79	1.56	20.03
B_2 (50-70)	1.9	5.10	4.57	ND	ND	ND	ND
BC (70-85)	1.4	5.00	1.78	ND	ND	ND	ND
Makino (No.62)							
A_{11} (0-8)	13.6	4.75	9.77	1.69	5.14	1.07	12.66
A_{12} (8-23)	13.1	4.70	25.56	1.81	7.66	1.32	18.87
A_{13} (23-38)	11.7	4.78	21.74	1.94	7.59	1.34	20.75
A_3 (38-56)	6.2	4.77	7.50	2.14	5.88	1.29	15.27
B_2 (56-75)	1.0	4.75	0.12	ND	ND	ND	ND
Godan (No.35)							
A_{11} (0-8)	18.3	4.60	29.08	1.48	1.18	0.78	0.96
A_{12} (8-16)	11.9	5.39	31.65	1.46	1.38	0.74	1.20
A_{13} (16-23)	9.9	4.92	37.12	2.02	2.36	1.15	2.39
A_3 (23-45)	5.7	5.61	25.23	1.79	1.93	0.80	2.39
B_2 (45-65)	2.2	5.55	7.97	2.01	1.73	0.89	3.42
B_3 (65-85)	1.1	5.20	2.98	3.73	1.17	1.03	0.75
Nekodake (No.12)**							
A_{11} (0-7)	21.5	3.85	4.13	2.13	1.55	1.54	1.72
A_{12} (7-30)	20.7	4.43	7.41	3.19	3.09	2.73	3.27
A_3 (30-38)	11.6	5.01	7.97	2.00	7.17	2.05	9.13
BC (38-60)	7.6	5.08	2.71	3.70	7.04	2.77	14.25
Red soils							
Motobu (No.43)							
A_1 (0-3)	3.8	4.23	0.87	1.47	0.33	0.70	0.16
B_{21} (3-10)	1.8	4.00	0.96	1.97	0.48	0.36	0.17
B_{22} (10-30)	0.8	4.55	0.91	3.60	0.82	0.18	0.25
B_3 (30-38)	0.5	4.58	0.08	4.81	0.79	0.04	0.03
Brown forest soils							
Nagamine (No.66)							
A_{11} (0-7)	9.1	4.27	19.22	1.41	0.56	0.46	0.59
A_{12} (7-15)	4.2	4.62	16.89	1.12	0.57	0.45	0.59
B_{21} (15-30)	1.6	4.62	4.09	1.61	0.77	0.64	0.97
B_{22} (30-55)	1.0	4.62	2.26	1.58	0.71	0.69	0.86
B_{23} (55-80)	0.7	4.89	0.56	1.59	0.70	0.67	0.78
BC (80-100)	0.5	5.18	ND	ND	ND	ND	ND

* See Section III-2.

** The data except for CLN contents were analyzed by Tamura and Oba [1986].

only Nagamine soil profile. Therefore, the contents of **CLN** were measured in soil samples taken from each 5 cm segment in A horizons (0-5, 5-10, 10-15cm) of Nagamine soil profile. As results, these soil samples (0-5, 5-10 and 10-15cm) contained 17.63, 23.43 and 10.83ppm of **CLN**, respectively. These facts suggests that **CLN** accumulatively distributes not in surface soils but in subsurface soils. However, in soil samples of each soil profile no relationships, between **CLN** content and the other chemical data were apparent as shown in Table III-3.1.

To compare with the distribution of **CLN** contents, the contents of 7,7'-biphyscion (**BP**) were estimated in same samples of each horizon of Makino soil profile. **BP** was the most prominent anthraquinone pigment in the surface soil, next to **CLN**, as described in Chapter II. As shown in Fig. III-3.4, the verti-

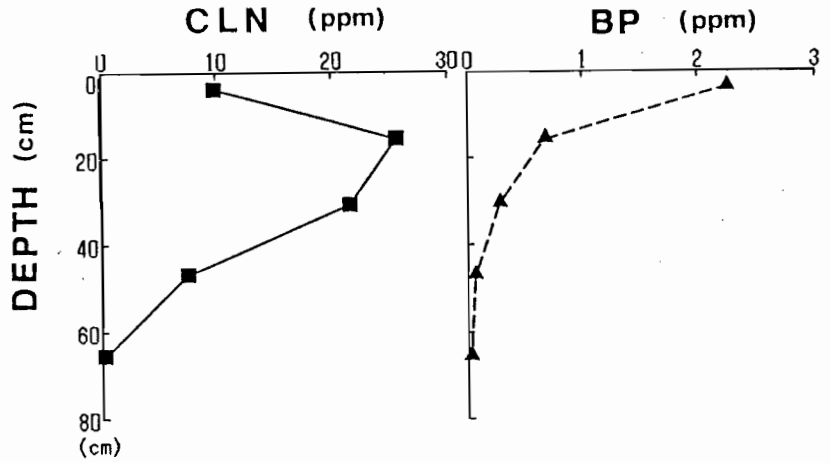


Fig. III-3.4. Distribution of **CLN** and **BP** contents in Makino soil profiles.

cal distribution of BP contents showed a rapid decrement with depth from 2.3ppm at the A_{11} horizons to 0.04ppm at the B_2. The distribution patterns of both similar compounds such as CLN and BP was not completely identity.

McGrath [1972] presumed from results of his soil column experiments that the vertical distribution pattern of CLN in podzolic soils probably resulted from non-uniform formation of CLN in the profile rather than from elution behavior in the profile. In the studies on the origin of CLN (see Section II-1), it was found that its origin was microorganisms. These facts may be ascribed to the formation of CLN in all horizons, in situ. However, high CLN contents in both B_2 horizons of Hachibuse-I and Godan soil profiles suggest that CLN is formed at a certain upper horizon and translocated downward by effective formation of complex of CLN and some minerals.

Contents of CLN in different humus fractions

To study on the relationship between CLN and humic substances, the CLN contents in HN, FA and HA fractions obtained from the profile of Hachibuse-II soil were determined. Although the CLN content in the A_{11} horizon of Hachibuse-II soil profile was not same level as those of Hachibuse-I soil profile. The distribution of CLN in both soil profiles tended to show the same pattern.

In the upper three horizons, the humus properties and the contents of CLN extracted from each humus fraction were listed

in Tables III-3.2 and -3.3, respectively. The total carbon contents in the A_{11}, A_{12} and A_3 horizon were 21.4, 11.2 and 6.1%, respectively. The distribution pattern of CLN contents in this soil profile did not reflect in the humus compositions and humus properties as shown in the table. The values of recovery in each horizon — the total contents of CLN extracted from three humus fractions to the normal extracted contents of CLN — went up to over 100%. The fact was probably resulted from the increment of extraction induced by fractionation operation.

Table III-3.2. Humus properties of A horizons in Hachibuse-II soil profile.

horizons (cm)	T-C (%)	Humus composition (%)			Δlog K	Rf	HA type
		HA	FA	HN			
A_{11}(0-17)	21.4	33.5	31.3	35.3	0.556	239	A
A_{12}(17-35)	11.2	29.6	40.2	30.2	0.496	227	A
A_3 (35-50)	6.1	28.0	40.1	32.0	0.518	174	A

Table III-3.3. CLN contents in different humus fractions of A horizons in Hachibuse-II soil profile.

horizons (cm)	CLN contents (ppm)*					recovery (%)
	HA	FA	HN	sum	soil	
A_{11}(0-17)	18.68	0.56	22.44	41.68	31.11	134.0
A_{12}(17-35)	22.31	0.21	21.50	44.02	37.18	118.4
A_3 (35-50)	9.03	0.11	24.27	33.41	31.01	107.7

* Expressed as in unfractionated soil.

As shown in Table III-3.3, little CLN was present in the FA fractions (<0.5ppm) in all horizons. The soil profile showed to be similar in CLN contents (at about 23ppm) in the HN fractions. These vales went up to over a half of the total CLN contents. On the other hand, the changes of the CLN contents in the HA fractions tend to reflect those of the total CLN contents in each fraction, as shown in Fig. III-3.5. Although the number of samples are not enough, it is suggests that the distribution of CLN in this soil profile may depend on that in the HA fractions.

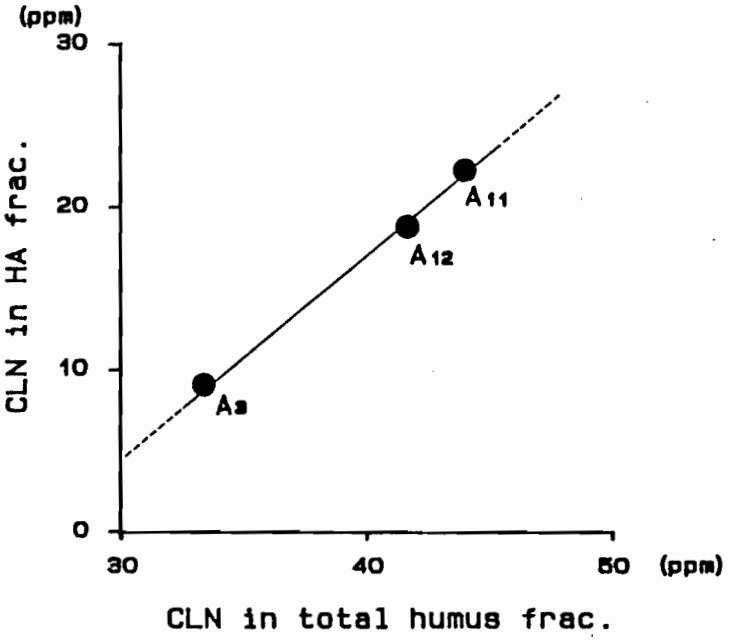


Fig. III-3.5. Relationship of CLN contents between in HA fraction and in total humus fractions obtained from Hachibuse-II soil profile.

Relation between CLN contents and iron or aluminum contents

The relationship between total CLN contents and CLN contents in HA fraction suggests that the presence of CLN in the soil may be influenced by that of certain mineral in HA fraction. According to Nakabayashi et al. [1982], the contents of Pg fraction, which is possible dihydroxyperylenequinone derivatives and has worldwide distribution in soils as well as CLN [Kumada, 1987], have the correlation with iron contents in HA fraction. Therefore, relationships between CLN contents and iron or aluminum contents in HA fraction were examined. As results, no correlations were found between them as shown in Table III-3.4.

As described above, attempts to relate CLN contents to the contents of DCB extractable iron and aluminum or of Tamm extractable iron and aluminum were unfruitful. However, it seems that the high accumulation of CLN in black soils may be resulted by complexing certain form of minerals (especially aluminum) with CLN (see Section III-2).

Table III-3.4. CLN contents and iron and aluminum contents in HA fractions of A horizons in Hachibuse-II soil profile.

horizons (cm)	CLN (ppm)	in HA fraction (ppm)*			
		CLN	Fe	Al	Fe+Al
A_{11}(0-17)	31.11	18.68	746	444	1190
A_{12}(17-35)	37.18	22.31	92	102	194
A_3 (35-50)	31.01	9.03	51	119	170

* Expressed as in unfractionated soil.

Therefore, iron and aluminum contents in the solution extracted successively with NaOH, $Na_4P_2O_7$ and $Na_4P_2O_7$+$NaBH_4$ were determined. Although the chemical spaces of iron and aluminum in each solution are not obvious, it is presumed that the stability of complexes between such minerals and organic matter is higher in order of $Na_4P_2O_7$+$NaBH_4$-extract > $Na_4P_2O_7$-extract > NaOH-extract. However, there were no relationships between these extractable iron or aluminum and CLN (Table III-3.5). Thus, it seems that the behavior of CLN may be correlated with clay or other minerals rather than with iron or aluminum.

Table III-3.5. Iron and aluminum contents (mg/g soil) extracted succesively with different solvents and CLN contents in A horizons of Hachibuse-II soil profile.

horizons (cm)	CLN (ppm)	NaOH		PPNa		PPNa+BHNa	
		Fe	Al	Fe	Al	Fe	Al
A_{11}(0-17)	31.11	3.23	22.00	1.31	29.49	2.44	27.46
A_{12}(17-35)	37.18	0.47	5.98	0.34	3.63	2.04	3.48
A_3 (35-50)	31.01	0.85	1.70	0.19	2.53	1.71	1.98

NaOH, 0.1N NaOH extractable; PPNa, 0.1M $Na_4P_2O_7$ extractable; and PPNa+BHNa, 0.1M $Na_4P_2O_7$+$NaBH_4$ extractable.

CLN contents in surface and buried humus horizons in soil profile

Table III-3.6 shows the contents of **CLN** in the surface and buried horizons in a soil profile of the cumulative volcanic ash. Little **CLN** was contained in the surface horizons (0-60cm) accumulated during the past 1,700 years. However, the contents of **CLN** in the buried humus horizons (about 17-20m), dating back 6,400 to 10,200 **YBP**, were much higher than those in the surface horizons. This fact suggests its high stability. The distribution of **CLN** in **HN** fractions also suggests its high stability.

It is possible that these **CLN** contents reflect the amounts originally accumulated in the horizon when this horizon was at

Table III-3.6. CLN contents in the surface and buried humus horizons of Fuji-Subashiri soil profile.

horizon	depth	AGE*(10^3YBP)	CLN (ppm)
Surface soil	0-10 (cm)	< 1.7	0.3
Hoei-scoria	10-60		0.1
Fuji-Kurotsuchi			
S-0-6	17.4-18.2 (m)	6.4	1.6
S-0-3	19.0-19.5	9.2	1.4
S-0-1	19.9-20.2	10.3	2.7

* The soil age was determined by several key horizons with known radio-carbon (^{14}C) ages [Uesugi and Yonezawa, 1987].

the surface or subsurface. Without any protection mechanism, the **CLN** in the buried horizons is impossible to persist during such a long period. It is therefore, considered that **CLN** must be stabilized by complexing with several soil components. Moreover, since **CLN** must be originated from microorganisms (see Section II-1), **CLN** may be regarded as an indicator reflecting the soil environment at the past time.

CONCLUSION

The presence of simple organic compounds in soils were influenced by interaction with soil minerals, microbes and other soil organics.

Nearly all of anthraquinones isolated from natural sources are polyhydroxy (methoxy) derivatives with little variation in skeletal structure, and the most conventional type is of substances with adjacent OH and O (e.g., 1-hydroxy-9,10-anthraquinone) [Thomson, 1987]. Undoubtedly, the substances of such structure type strongly chelate with polyvalent cations (Fig. IV-1). For example, Engstrom et al [1980] demonstrated iron chelating capability of PYS in vitro. All of soil hydroxyanthraquinones as shown in Fig. IV-2., belong to the type of 1,8-dihydroxy group. Therefore, the behavior of hydroxyanthraquinones in soils is of interest for understanding interaction between soil organic and mineral components.

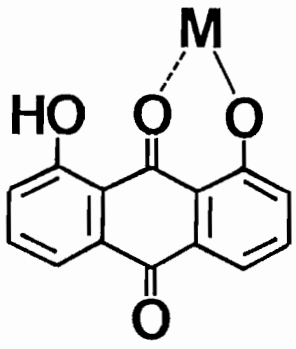
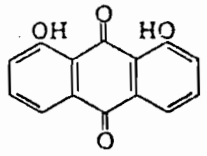
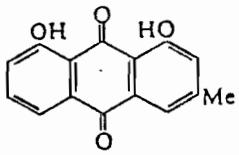


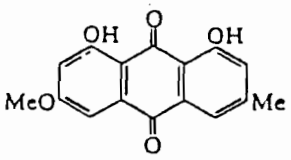
Fig. IV-1. Chemical structure of hydroxyanthraquinone-metal complex.



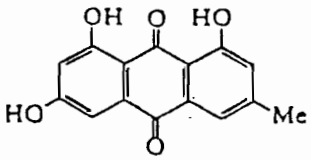
chrysazin



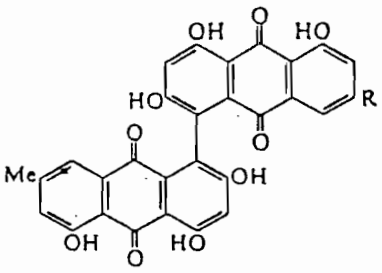
chrysophanol



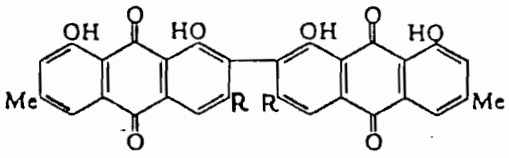
physcion



emodin



R=Me, skyrin
R=CH_2OH, oxyskyrin



R=H, chrysotalunin
R=OMe, 7,7'-biphyscion

Fig. IV-2. Chemical structure of soil hydroxyanthraquinones.

On the other hands, the importance of this compounds in humus chemistry have been deduced from several reasons: (i) It is well-known that the substances of such structure type form stable free radicals in some conditions (Fig. IV-3), and it is speculated that a radical may catalyze polymerization of soil organic compounds [McLaren and Peterson, 1967]. (ii) Since soil humic polymers may be constituted of polynuclear aromatics, it was considered that hydroxyanthraquinones may be one of the constituent of soil humic substances.

However, there are only a few reports on the occurrence of soil hydroxyanthraquinones, and information on their distribu-

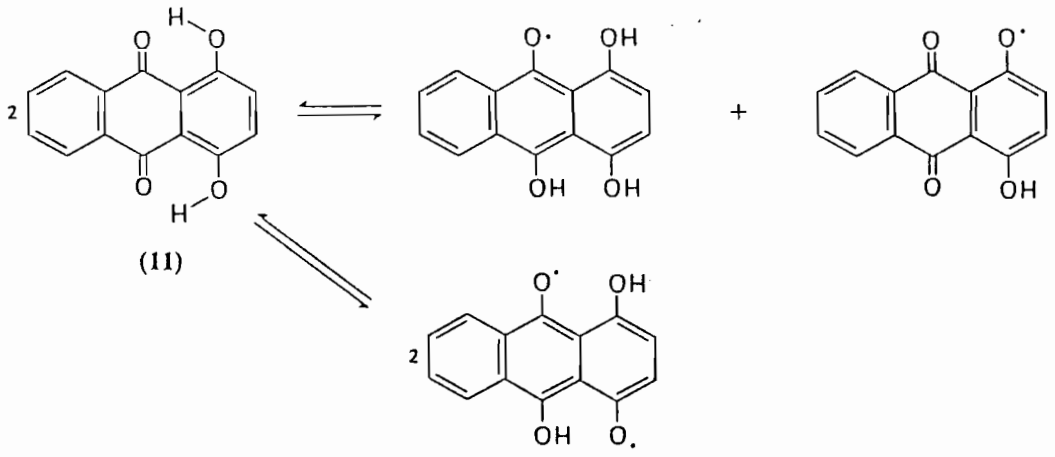


Fig. IV-3. Formation of mono- and bi-radicals from hydroxyanthraquinones.

tion, behavior and role in soil is insufficient and fragmentary.

Many findings as described in Chapters II and III were given in this studies. Particular interests were as follows:

1) The major soil hydroxyanthraquinones are bianthraquinones such as **CLN** and its derivatives (**BP** and possible pigment **D**), rather than monomeric hydroxyanthraquinones (e.g., **EMD**, **CPL** and **PYS**).

2) Chrysotalunin is the most prominent soil hydroxyanthraquinones, distributes universally in Japanese soils, and has a characteristic distribution pattern in soil profiles.

EMD and other monomers detected in soils were the most numerous compounds in naturally occurring quinones obtained from a number of biosis. Despite this, bianthraquinones are minor compounds in those even if many more bianthraquinone await discovery. Most, if not all, of soil hydroxyanthraquinones originate from microorganisms as described in Chapter II. Therefore, such curious features may be brought by the productivity of these compounds by microorganisms. However, according to McGrath [1972], his examination on **CLN** and **CPL** incubated with a soil suggests that **CLN** has the much higher resistance to chemical and/or biological degradation in soils than **CPL**. Moreover, since **CLN** and **BP** are extremely low solubility in water, it is likely that these bianthraquinones are much less biodegradable than other soil anthraquinones. Thus, the

abundance and composition of soil hydroxyanthraquinones must be influenced by their resistance to biodegradation and/or by their adsorptive capability on soil minerals.

On the other hands, the findings of characteristic distribution of **CLN** in soil profiles are of very interest for understanding on dynamics of simple organic compounds in soils. Although the definitive factors influencing such curious features could not be found, it was deduced that the occurrence of **CLN** may be resulted by activity of microorganisms in soils and its accumulation may be resulted by the complexing of **CLN** with soil mineral components. Especially, the findings that **CLN** accumulatively distributes in subsurface horizons and that the similar compound **BP** has a different distribution in a same profile suggests that difference of the chemical properties of these compounds affect their behavior in soil. Furthermore, the presence of **CLN** in buried horizons suggests that **CLN** may be regarded as an indicator reflecting the soil environment or the ecology of soil microorganisms for the past time.

Therefore, it is likely that clarification of the behavior (distribution) of the individual soil hydroxyanthraquinones leads to appearance of a new indicator in order to characterize the soil environment, and to search the lives of soil microorganisms.

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