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Investigation of the Neutral Constituents of *Lythrum Salicaria* L

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Isolation and characterization of di-isobutyl, *n*- and *iso*-butyl, and di-*n*-butyl phthalate, β-sitosterol, and loliolide from the methanol extract of the whole herb of *Lythrum Salicaria* L are described. Detections of dioctyl phthalate, four new diheptyl phthalates, and two new dinonyl phthalates by the combined gas chromatography-mass spectrometry are also described.

We have investigated the alkaloids of *Lythrum anceps* MAKINO (Japanese name: "misohagi").[1] Now, we carried out an investigation on the neutral constituents of *Lythrum Salicaria* L (Japanese name: "ezo-misohagi"), which was found to contain very little alkaloidal constituents.

The methanolic extract of the plant material collected in the suburbs of Sapporo was separated into neutral, basic, phenolic, and acidic fractions as described in the experimental section. The neutral fraction was chromatographed on silica gel column. Elution with benzene gave a viscous oil (I), and the following elutions with a mixture of benzene and methylene chloride (1 : 1) and with methylene chloride afforded colorless prisms of m.p. 134–137° (II) and colorless plates of m.p. 154–156° (III), respectively.

The viscous oil (I) was pale yellow and showed the infrared (IR) absorption bands at 1725, 1290, 1120 (ester group(s)), 1597, 1580, 1470, 1075, 1040, and 700 cm^{-1} (aromatic ring). Its nuclear magnetic resonance (NMR) spectrum in deuteriochloroform gave the following signals: terminal methyls at δ 0.8–1.0, methylenes adjacent to ester oxygen at δ 4.0–4.5, and aromatic protons at δ 7.6 (symmetric multiplet). These spectral data led to the conclusion that the major constituents of the oil I were alkyl phthalates. The combined gas chromatography-mass spectrum (GC-MS) of oil I showed that it contained ten alkyl phthalates. Thus, its GC showed ten peaks (1∼10) (Fig. 1), and the MS of each peak showed the fragment ion peaks at m/e 149 and 167, which were characteristic of alkyl phthalate and assignable to structures, **1** and **2**[2], respectively.

The mass spectra of the GC peaks 1, 2, and 3 showed the common fragment ion peaks at m/e 223, 205, 167, 149, 57, and 43. Structures **4a** and **3a** were assigned to the fragment ions which corresponded to the peaks at m/e 223 and 205, respectively. Thus, it was clarified that the substances of GC peaks 1, 2, and 3 were isomeric dibutyl phthalates. Previously, Matsuura *et al.*[3] published the occurrence of di-isobutyl, *iso*- and *n*-butyl, and di-*n*-butyl phthalates in *Cryptotaenia canadensis* DC. *var japonica* Makino (Japanese name:

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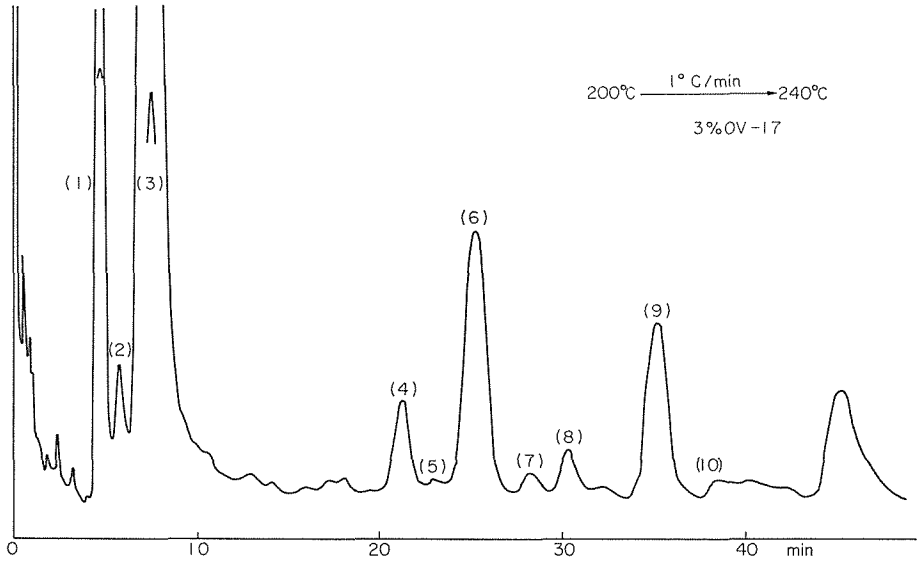
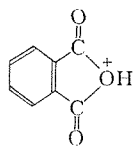


Fig. 1. The Gaschromatogram of oil I.



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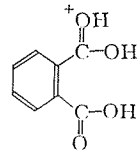

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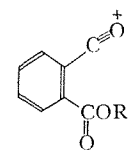
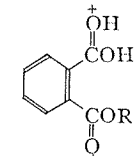


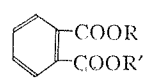
3a R=C_4H_9
3b R=C_7H_{15}
3c R=C_8H_{17}
3d R=C_9H_{19}



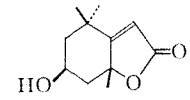
4a R=C_4H_9
4b R=C_7H_{15}
4c R=C_8H_{17}
4d R=C_9H_{19}

"mitsuba"). We compared the GC and MS of their authentic samples* with those of the GC peaks 1, 2, and 3 substances (Table 1). Consequently, it was concluded that the peaks 1, 2, and 3 corresponded to di-isobutyl (**5**), *iso*- and *n*-butyl (**6**), and di-*n*-butyl phthalates (**7**), respectively.[4] Moreover, the peak 3 substance was isolated as a pure compound *via* repeated silica gel column chromatography, and its structure was confirmed by the IR, UV, and NMR investigations. Its hydrolysis afforded phthalic acid as expected.

The GC peaks 4 to 7 (Fig. 1) had the common fragment ion peaks of m/e 362 (M^+), 265 assignable to **4b**, and 247 assignable to **3b** in their mass spectra, suggesting them to be



5 R=R′=$-CH_2CH(CH_3)_2$
6 R=$-CH_2CH(CH_3)_2$
R′=$-(CH_2)_3CH_3$
7 R=R′=$-(CH_2)_3CH_3$
8 R=R′=C_7H_{15}
9 R=R′=C_8H_{17}
10 R=R′=C_9H_{19}



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* Only *iso*- and *n*-butyl phthalate was synthesized by us (See experimental section). The other two phthalates were commercially available.

Table 1. Comparison of Mass Spectra of GC Peaks 1 to 3 with Those of Authentic Samples.

m/e	278[a]	223(**4a**)	205(**3a**)	167(**2**)	149(**1**)	$57C_4H_9^+$	$43C_3H_7^+$
di-iso[b]	—	5.2	1.7	4.3	100	30.8	8.9
iso-n[b]	—	5.2	3.1	2.3	100	16.0	7.7
di-n[b]	0.3	3.3	3.3	0.6	100	5.8	3.6
peak 1[c]	—	5.9	2.0	7.6	100	33.2	8.0
peak 2[c]	—	5.9	3.5	3.5	100	18.8	10.2
peak 3[c]	—	4.4	4.4	0.4	100	5.8	2.3

Relative peak heights reffered to base peak of spectrum as 100.

a Molecular ion peak.

b *Authentic samples of butyl phthalates.*

c See Fig. 1.

the isomers of di-heptyl phthalates (**8**). The mass spectrum of the GC peak 8 substance showed no molecular ion peak, but the fragment ions at m/e 279 and 261, which were assigned to structures **4c** and **3c** respectively, suggested it to be dioctyl phthalate (**9**). Subsequently, the mass spectra of the peaks 9 and 10 of Fig. 1 gave the common molecular ion peak at m/e 418 and the common fragment ion peaks at m/e 293 and 275, which were assigned to **4d** and **3d** respectively, suggesting them to be the isomers of dinonyl phthalate (**10**).

The crystalline substance II gave a positive Liebermann-Burchard test and its MS (M^+ 414) and GC clarified it to be β-sitosterol contaminated by a trace of unidentified compound.

The crystalline component III had $[\alpha]_D$-107°. Its MS had the molecular ion peak at m/e 196. The IR and UV spectra suggested the presence of an α,β-unsaturated five-membered lactone ring, and the NMR spectrum indicated the proton signals attributed to three tertiary methyl groups and an olefinic proton signal as described in the experimental section. Acetylation of this compound gave a monoacetate, m.p. 88–88.5°. These facts led to an assumption that it might be the known loliolide (**11**),[5] which was proved to be correct by its direct comparison with the authentic sample.

The occurrence of diheptyl and dinonyl phthalates in nature is the first finding.

EXPERIMENTAL

Melting points were determined on a micro hot-stage and were uncorrected. The IR and UV spectra were determined with a Hitachi EPI-S_2 Spectrometer and a Hitachi EPS-3 Spectrophotometer, respectively. The NMR spectra were taken with a Varian A-60 (at 60 MC) spectrometer for the $CDCl_3$ solutions. The specific rotations were measured with a JASCO DIP-180 automatic polarimeter. A Hitachi K-53 Gaschromatograph and a Hitachi RMU-6E single-focused Mass Spectrometer were used for the combined GC-MS.

Extraction and separation. The plant materials used in this investigation were collected in the suburbs of Sapporo, during August 1970. To 1.43 kg of methanolic extract prepared from 5.4 kg of the dried whole herbs was added 8 l. of 0.5% HCl. After the

mixture had been left for 3 days, it was filtered through a Celite bed. The filtrate was extracted with ether repeatedly. Usual treatment of the aqueous layer yielded little alkaloids. The ethereal layer was shaken with 10% Na_2CO_3 and 10% NaOH to remove acidic and phenolic substances, washed with water, dried over anhydrous sodium sulfate, filtered and then evaporated *in vacuo* to give 2.1 g of viscous neutral material. The residue filtered from the foregoing 0.5% HCl extract was extracted with methylene chloride. The filtrate from the residue, after shaking with 10% NaOH and 1% HCl to remove acidic and basic substances, was treated as usual to give 62 g of viscous neutral material. The above two viscous neutral materials were mixed and chromatographed on 800 g of Kiesel gel (Merck) column. The following substances were eluted with benzene, benzene-methylene chloride and methylene chloride in order.

Alkyl phthalates. The first fraction eluted with benzene was rechromatographed on silica gel column. Elution with benzene gave 330 mg of pale yellow viscous oil (I). The combined GC-MS of the oil (I) gave the following data (see Fig. 1). The GC peaks 1, 2, and 3 gave the common fragment ions at m/e 223 (**4a**), 205 (**3a**), 167, 149, 57, and 43. The molecular ion peak appeared at m/e 278 in the MS of GC peak 3. Di-*n*-buthyl phthalate (peak 3) was isolated by column chromatography on silica gel using benzene as eluent. IR $\nu_{max.}^{CHCl_3}$ cm^{-1} 1720, 1290, 1120 (-C(=O)-O-), 1597, 1580, 1467 (aromatic ring), UV $\lambda_{max.}^{95\%EtOH}$ nm: 224, 276, 284. NMR ($CDCl_3$) δ_{ppm}: 0.96 (6H, triplet, J=6 Hz, 2×$-CH_2CH_3$) 4.30 (4H, triplet, J=6.3 Hz, 2×$-COOCH_2CH_2$) 7.61 (4H, symmetrical multiplet, 4×aromatic H) MS m/e 278 (M^+ peak). The hydrolysis of this compound using 1% methanolic KOH solution gave a crystalline compound, m.p. 225°, which was proved to be identical with phthalic acid (m.p., m.m.p., IR). The mass spectra of the GC peaks 4, 5, 6, and 7 gave the fragment ions at m/e 362 (M^+ peak), 265 (**4b**), 247 (**3b**), 167, and 149 (base peak). The mass spectrum of the GC peak 8 gave the fragment ions at m/e 279 (**4c**), 261 (**3c**), 167, and 149 (base peak). The molecular ion peak was absent. The mass spectra of the GC peaks 9 and 10 exhibited the fragment ion peaks at m/e 418 (M^+), 293 (**4d**), 275 (**3d**) 167, and 149 (base peak).

Synthesis of iso- and n-butyl phthalate. *n*-Butyl phthalate was prepared by the procedure of Gogans.[6] This compound (5 mol) was mixed with isobutyl alcohol (1 mol), and the mixture was refluxed under the presence of *p*-toluene sulfonic acid for 12 hr. The remaining unchanged isobutyl alcohol was evaporated off *in vacuo* to leave a residue, which was chromatographed on a silica gel column to give the desired *iso*- and *n*-butyl phthalate. The purity was checked and confirmed by GC. NMR ($CDCl_3$) δ_{ppm}: 4.03 (2H, doublet, J=6.2 Hz, $COOCH_2CH(CH_3)_2$), 4.32 (2H, triplet, J=6.0 Hz, $COOCH_2CH_2$), 7.63 (4H, sym. multiplet, arom. protons).

β-Sitosterol. The fractions eluted with benzene-methylene chloride mixture (1 : 1) were rechromatographed on silica gel, and 1.2 g of a crude crystalline substance was obtained from acetone. It was rechromatographed on Woelm neutral alumina column. The fraction eluted with benzene-methylene chloride (7 : 3) afforded a crystalline mass which was recrystallized from methanol to give 525 mg of flat plates, m.p. 134~137°. By the gas chromatographic comparison with the authentic sample, the crystalline compound was proved to be β-sitosterol contaminated by a trace of the unknown compound.

Loliolide (11). The fractions eluted with methylene chloride yielded 39 mg

of a crude crystalline substance. Recrystallization from benzene yielded colorless flat plates, m.p. 154~156°, $[\alpha]_D$—107° (c=0.06, $CHCl_3$). Mass spectrum m/e : 196 (M^+). IR $\nu_{max.}^{KBr}$ cm^{-1}: 3370(-OH), 1730, 1720, 1275(-CO-), 1620(-C=C-). UV $\lambda_{max.}^{95\%EtOH}$ 213 nm (ϵ=13,200). NMR ($CDCl_3$) δ_{ppm}: 1.27, 1.47, 1.78 (each 3H, singlet, $-CH_3$), 4.33 (1H, quintet, J=3.6 Hz, -C*H*-OH), 5.68 (1H, s, -C=CH-). This compound was proved to be identical with an authentic sample of loliolide (m.p., m.m.p., and IR).

Loliolide acetate. Fifteen mgs of loliolide was acetylated using acetic anhydride and pyridine, and recrystallization from *n*-hexane-chloroform yielded 10 mg of loliolide acetate: m.p. 88~88.5°. *Anal.* Calcd. for $C_{13}H_{18}O_4$: C, 65.53; H, 7.61. Found: C, 65.66; H, 7.72. IR $\nu_{max.}^{KBr}$ cm^{-1} : 1740, 1630, 866. NMR ($CDCl_3$) δ_{ppm} : 1.30, 1.41, 1.72 (each 3H, singlet, CH_3), 2.10 (3H, singlet, $-OCOCH_3$), 5.25 (1H, quintet, J= 3Hz, -CH-OAC), 5.73 (1H, singlet, -C=CH-). These spectral data were identical with those cited in the literature.[5]

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REFERENCES

(1) a) E. Fujita, K. Fuji, K. Bessho, A. Sumi and S. Nakamura, *Tetrahedron Letters*, 4595 (1967); b) E. Fujita, K. Bessho, K. Fuji and A. Sumi, *Chem. Pharm. Bull.* (Tokyo), **18**, 2216 (1970); c) E. Fujita, K. Fuji, K. Bessho and S. Nakamura, *ibid.*, **18**, 2393 (1970); d) E. Fujita, K. Fuji and K. Tanaka, *Tetrahedron Letters*, 5905 (1968): *idem., J. Chem. Soc.* (*C*), 205 (1971): e) E. Fujita and K. Fuji, *J. Chem. Soc.* (*C*), 1651 (1971): f) E. Fujita, K. Bessho, Y. Saeki, M. Ochiai and K. Fuji, *Lloydia*, **34**, 306 (1971): g) E. Fujita and Y. Saeki, *J. Chem. Soc.* (*D*) (*Chem. Comm.*), 368 (1971); h) *idem., Chem. Pharm. Bull.* (Tokyo), **19**, 1515 (1971); i) *idem., J.C.S. Perkin I*, in press.

(2) E. M. Emery, *Anal. Chem*, **32**, 1495 (1950).

(3) S. Hayashi, Y. Asakawa, T. Ishida and T. Matsuura, *Tetrahedron Letters*, 5061 (1967).

(4) F. W. McLafferty and R. S. Gohlke, *Anal. Chem*, **31**, 2076 (1956).

(5) R. Hodges and A.L. Porte, *Tetrahedron*, **20**, 1463 (1964); T. Wada, *Chem. Pharm. Bull.* (Tokyo), **13**, 43 (1965).

(6) J. Gogans, *J. Amer. Chem. Soc.*, **61**, 2909 (1939).