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33. Hiroshi Mitsuhashi and Taro Nomura : Studies on the Constituents of Asclepiadaceae Plants. XV.*1 On the Components of *Metaplexis japonica* MAKINO. II.*2

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It was reported in the preceeding paper that the stems and leaves of *Metaplexis japonica* MAKINO (Asclepiadaceae) contain a glycoside mixture, from which D-cymarose and D-digitoxose were identified as the sugar components. Sarcostin (I), metaplexigenin (II), and three other aglycones were isolated from the aglycone mixture.[1]

In the present paper, some later findings on the components of the roots, and the structure of metaplexigenin (II) are described. Successive percolation of the powdered roots with chloroform and removal of solvent afforded a powdery extract which showed a strong Keller-Kiliani reaction (bluish violet), suggesting a presence of a glycoside containing 2-deoxysugar components, same as from the stems and leaves.[1] The crude glycoside, thus obtained, showed positive Keller-Kiliani and Liebermann-Burchard reactions, and a positive antimony trichloride test. When the glycoside mixture was hydrolysed with 0.05*N* sulfuric acid in 50% methanol, the same condition applied to the hydrolysis of cardiac glycoside containing 2-deoxysugars,[2] and after removal of methanol, ether and chloroform extracts gave negative Keller-Kiliani reaction, but showed positive Liebermann-Burchard, and antimony trichloride tests. These facts suggested the presence of steroidal ester glycosides containing 2-deoxysugars, as shown in the preceeding paper.[1,3,4] The thin-layer chromatographic study of these extracts (Fig. 1) showed a number of unknown spots besides metaplexigenin (II). Chromatography of the mixture through alumina yielded four crystalline substances. Two of them were β-sitosterol and metaplexigenin (II), which were also obtained from the stems and leaves.[1] One of the remaining two (III) showed m.p. 272~275°, for which molecular formula $C_{28}H_{36}O_5$ was proposed from its elemental analysis. With a Liebermann-Burchard reaction, III gave color change pink→blue→orange, a reddish violet with antimony trichloride, and a negative Keller-Kiliani reaction. This compound (III) was named benzoylramanone,*4 and the structure will be discussed in the following paper of this series. The other compound (IV) showed m.p. 192~197°, which with a Liebermann-

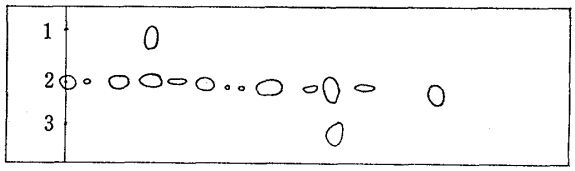

Fig. 1. Example of Thin-layer Chromatographic Separation of Esteraglycone Mixture

System : $CH_3COC_2H_5$-benzene (3:7), Al_2O_3
1 : Metaplexigenin
2 : Esteraglycone mixture
3 : Benzoylramanone

*1 Part XIV : This Bulletin, **13**, 267 (1965).

*2 A part of this work was reported at 82nd Annual Meeting of the Pharmaceutical Society of Japan, Nov. 3, 1962, Shizuoka, and the preliminary report of this paper was published previously (H. Mitsuhashi, T. Nomura : This Bulletin, **11**, 1333 (1963)).

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*4 "Rama" is the Chinese name for *Metaplexis japonica* MAKINO.

1) H. Mitsuhashi, T. Nomura, Y. Shimizu, I. Takemori, E. Yamada : This Bulletin, **10**, 811 (1962). In this report, metaplexigenin was referred to as crystal 5.
2) T. Reichstein, *et al.* : Helv. Chim. Acta, **37**, 737 (1954).
3) H. Mitsuhashi, Y. Shimizu : This Bulletin, 8, 313 (1960).
4) H. Mitsuhashi, I. Takemori, Y. Shimizu, T. Nomura, E. Yamada : This Bulletin, **10**, 804 (1962).

Burchard reaction gave color changes reddish violet→green, green with antimony trichloride, and a negative Keller-Kiliani reaction. Ⅳ analyzed for $C_{28}H_{36}O_7$ or $C_{28}H_{38}O_7$ and absorption maximum at 229 mμ (log ε 4.19) and 273 mμ (log ε 3.10). Infrared absorption maximum occurred at 3300, 3200, 1725, 1685, 1600, 1590, 1280 cm^{-1} and were assigned to hydroxyls, aromatic double bond, and ester, respectively. These facts highly suggest that Ⅳ is a monobenzoic acid ester. The hydrolysis of Ⅳ with 5% methanolic potassium hydroxide, followed by successive extraction of the neutral portion gave crystals m.p. 257~266° (Ⅴ). The acid fraction gave crystals, m.p. 121°, which were identified as benzoic acid by the comparison with an authentic sample. The structure determination of Ⅳ is now in progress.

Metaplexigenin (Ⅱ) forms needles, m.p. 268~275°, $[\alpha]_D^{14} = -80°$ (c=0.15, pyridine), for which the molecular formula $C_{23}H_{34}O_7$ was proposed from its elemental analysis and molecular weight determination (419, Rast). It gave a yellow color with tetranitro-

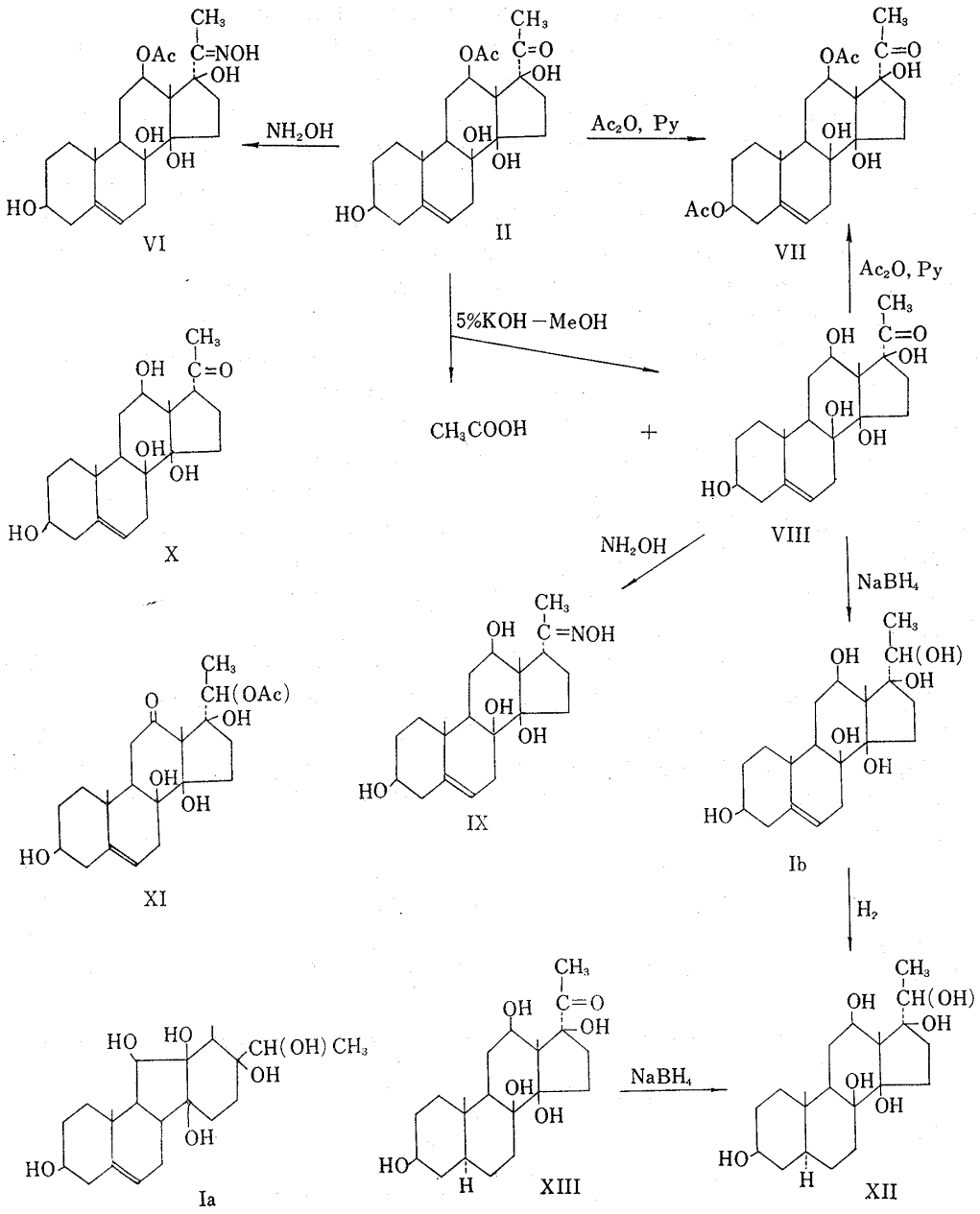

Chart 1.

methane. The infrared peaks at 3550, 3450, 3300, 1745, 1720, 1240 cm^{-1} show the presence of hydroxyl groups, carbonyl (six membered or open chain keton), ester (acetate), respectively. II would not form a semicarbazone, and gave only a monoxime (VI), m.p. 267° (decomp.), $C_{23}H_{35}O_7N$. Acetylation of II with acetic anhydride in pyridine yielded a monoacetate (VII), m.p. 262~264°, $C_{25}H_{36}O_8$, which showed hydroxyl absorption at 3550 (shoulder), 3460 cm^{-1}. Hydrolysis of II with 5% methanolic potassium hydroxide gave an acidic substance, which gave only one spot on paper chromatography (1.5*N* NH_3/BuOH) and was identified as acetic acid. A neutral product, deacylmetaplexigenin (VIII) was obtained as colorless plates, m.p. 218~223°, which was formulated as $C_{21}H_{32}O_6$ from its elemental analysis. It showed two bands at 1725, and 1690 cm^{-1} in the carbonyl region of its infrared spectra, but afforded only a mono oxime (IX) of m.p. 265° (decomp.), $C_{21}H_{33}O_6N$, which showed no infrared absorption in this area. These facts suggested the existence of only one carbonyl group. Acetylation of VIII with acetic anhydride in pyridine gave a diacetate, m.p. 255~261°, whose identity with monoacetyl metaplexigenin (VII) was confirmed by mixed fusion and comparison of infrared spectra. This indicates that the monoacetate of VIII is metaplexigenin (II), and no steric changes had occured during alkaline hydrolysis. Metaplexigenin (II) consumed one mole of lead tetraacetate (in dioxane, 164 hr.), and deacylmetaplexigenin (VIII) consumed about two moles of the reagent; one mole rapidly (40 min.), but last one mole very slowly (192 hr.). Sarcostin (I) consumed three moles, two moles rapidly, one mole slowly, and deacylcynanchogenin (X) consumed two moles of the reagent rapidly.[5] We are not able to reach a reasonable explanation for these results. These observation on II and VIII might be interpreted as follows: metaplexigenin (II) has one carbonyl group, two secondary hydroxyl groups (one hydroxyl present as an acetate) and three tertiary hydroxyl groups, and two hydroxyl groups out of the five exist as a glycol unit. Deacylmetaplexigenin (VIII) was reduced with sodium borohydride, and the product examined by paper chromatography ($CHCl_3$/formamide),[6] giving two spots. The major spot was identical with that of sarcostin (I). Trituration of the reaction products with acetone gave a pure crop of sarcostin (I), confirmed by mixed melting point, infrared spectrum, and paper chromatography. Sarcostin (I) was first isolated by Cornforth[7] and later reported from several plants of Asclepiadaceae family.[8] Sarcostin was tentatively formulated as Ia by Cornforth,[9] but recently, almost at the same time Reichstein, *et al*,[10] and Mitsuhashi and Shimizu[11] independently proposed the formula (Ib). The difference between sarcostin (I) and deacylmetaplexigenin (VIII) is only that between a hydroxyl group and carbonyl group. On the basis of formula (Ib), the structure of metaplexigenin (II) would be represented as a 12-acetoxy-20-oxo (II) or a 20-acetoxy-12-oxo (XI) compound. A 3-keto structure was excluded, since metaplexigenin supposely was an aglycone of a glycoside. The nuclear magnetic resonance spectra and optical rotatory dispersion curve was measured in order to make a choice between structures (II and XI). The nuclear magnetic resonance spectrum of metaplexigenin was measured in pyridine and is given in Fig. 2 and Table I. This spectrum showed four singlets at 8.57 (3H), 8.05 (3H), 7.90 (3H), 7.50 (3H) τ.*5 If metaplexigenin has formula (XI), the nuclear magnetic resonance should show a doublet from the coupling of the 21-CH_3 with the 20-hydrogen, for example, sarcostin

*5 In this paper, 10−p.p.m. value (from tetramethylsilane, used as internal standard) is used as τ.

5) H. Mitsuhashi, Y. Shimizu: This Bulletin, **10**, 719 (1962).
6) H. Mitsuhashi, Y. Shimizu, E. Yamada, T. Takemori, T. Nomura: *Ibid.*, **10**, 808 (1962).
7) J. W. Cornforth, J. C. Earl: J. Chem. Soc., **1939**, 737; **1940**, 1443.
8) E. Abish, T. Reichstein: Helv. Chim. Acta, **45**, 2090 (1962).
9) J. W. Cornforth: Chem. & Ind. (London), **1959**, 602.
10) K. A. Jaeggi, E. K. Weiss, T. Reichstein: Helv. Chim. Acta, **46**, 694 (1963).
11) H. Mitsuhashi, Y. Shimizu: Steroids, **2**, 373 (1963).

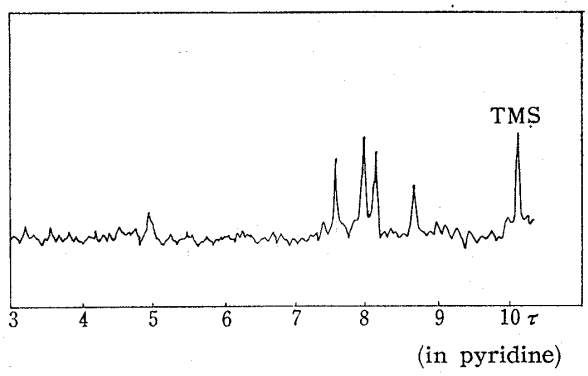

Fig. 2.

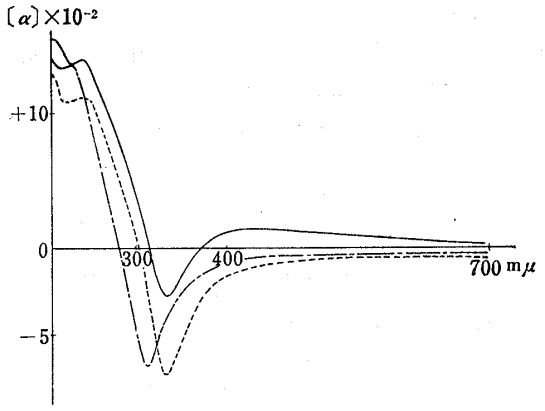

Fig. 3.

----- Metaplexigenin
——— Deacylmetaplexigenin
—·—·— Deacylcynanchogenin (in CH_3OH)

(I) shows a doublet at 8.53 τ (in pyridine).[11] But the spectrum of metaplexigenin lacks a signal in this region. The 17-$COCH_3$ signal of metaplexigenin appears at 7.50 τ and is similar to that of deacylcynanchogenin (X). The optical rotatory dispersion curves of metaplexigenin and deacylmetaplexigenin in methanol showed a negative Cotton effect (Fig. 3). These curves were shifted about 13 mμ to longer wave lengths compared to that of X, suggesting the presence of a α-ketol system. In the case of 17α-hydroxypregnan-20-one, the trough was shifted about 12.5 mμ to longer wave lengths (Table II).[12,13]

Table I.

Compound	Solvent	Signal (τ)			
		19-CH_3	18-CH_3	21-CH_3	-$OCOCH_3$
II	pyridine	8.57 (s)	8.05 (s)	7.50 (s)	7.90 (s)
Ib[11]	〃	8.59 (s)	8.11 (s)	8.53 (s)	
X[11]	〃	8.58 (s)	8.07 (s)	7.60 (s)	

Table II.

Compound	$\lambda_{m\mu}$	λ_{OH}
II	315	+12.5
VIII	316	+13.5
X	302.5	
XII[10]	320	+17.5
3β-Hydroxypregnan-20-one	307.5	
3β,17α-Dihydroxypregnan-20-one	320	+12.5

Moreover, it is well known that the Cotton effects of 12-oxosteroids are positive in C/D *trans* and C/D *cis* ring junctures.[13] A combination of the above results suggests structure (II) for metaplexigenin. Deacylmetaplexigenin (VIII) was isolated from *Cynanchum caudatum* by Mitsuhashi, *et al.*[14]

12) C. Djerassi: "Optical Rotatory Dispersion Application to Organic Chemistry," McGraw-Hill Book Co., 1960.
13) C. Djerassi, O. Halpern, V. Halpern, O. Shindler, Ch. Tamm: Helv. Chim. Acta, **41**, 250 (1958).
14) H. Mitsuhashi, Y. Shimizu, T. Nomura, T. Yamada, E. Yamada: This Bulletin, **11**, 1198 (1963).

Reichstein, *et al* obtained from African Asclepiadaceae plant, *Gongronema taylorii* (Schlter., et Rendle) Bullock, a compound $C_{21}H_{34}O_6$, similar to deacylmetaplexigenin (Ⅷ), which they named tayloron. Reduction of this compound with sodium borohydride gave dihydrosarcostin (Ⅻ), and from these results structure (XIII) was proposed.

Experimental

Extract——The root of *Metaplexis japonica* Makino, gathered at Kotoni, a suburb of Sapporo in July 1961, was chipped, dried, and powdered. 4.2 kg. of ground dry root was percolated with $CHCl_3$ at room temperature, and after removal of solvent 140 g. of a faintly yellow powder was obtained which was reprecipitated with hexane to remove oily substances. The hexane-insoluble precipitate was dissolved in MeOH. MeOH was evaporated *in vacuo* and the yellow colored powder (75 g.) was obtained. This powder appeared to be a glycoside mixture from color tests; Keller-Kiliani reaction (deep blue), Liebermann-Burchard reaction (red→green).

Hydrolysis of the Glycoside——A solution of 25 g. of the crude glycoside in 660 ml. of MeOH and 330 ml. of 0.15*N* H_2SO_4 was refluxed for 25 min. The MeOH was evaporated *in vacuo* at room temperature and the residue was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with 5% $NaHCO_3$ solution and H_2O, and dried over Na_2SO_4. Removal of the solvent gave 13 g. of a powder, Keller-Kiliani reaction: negative. By thin-layer chromatography (Fig. 1), about fourteen spots were detected. 35 g. of this residue was submitted to chromatography over Al_2O_3 (1 kg.). The results shown in Table Ⅲ.

Metaplexigenin (II)——Fraction Nos. 291～350 were recrystallized from acetone to give needles, m.p. 265～269° (200 mg.). Mixed melting point with crystal No. 5, obtained from stems and leaves,[1] had no depression. *Anal.* Calcd. for $C_{23}H_{34}O_7$: C, 65.38; H, 8.11. Found: C, 65.44; H, 8.14.

Table Ⅲ.

Fract. No.	Solvent (ml.)	Eluted product (g.)	Note
1～75	$CHCl_3$	8.69	β-sitosterol (300 mg.)
76～90	$CHCl_3$–MeOH (99.8 : 0.2)	2.80	benzoylramanone (540 mg.)
91～104	〃 (99.8 : 0.2)	1.22	powder
105～275	〃 (99.7 : 0.3)	5.56	〃
276～290	〃 (99.5 : 0.5)	0.29	〃
291～350	〃 (99.5 : 0.5)	0.65	metaplexigenin (200 mg.)
351～385	〃 (99.5 : 0.5)	0.29	powder
386～435	〃 (99 : 1)	1.19	〃
436～526	〃 (95 : 5)	1.82	Ⅳ (30 mg.)
527～545	〃 (4 : 1)	0.96	oil

one fraction 200 ml.

Benzoylramanone (III)——Fraction Nos. 76～90 were recrystallized from MeOH–H_2O to give needles, m.p. 222～226° (total 540 mg.). It showed reddish pink→blue→green→orange with a Liebermann-Burchard reaction, and reddish violet color with $SbCl_3$, and gave a negative Kedde and Legal test. *Anal.* Calcd. for $C_{28}H_{36}O_5$: C, 74.30; H, 8.02. Found: C, 74.28; H, 7.84. UV λ_{max}^{EtOH} mμ (log ε): 233 (4.11), 276 (3.18), 283 (3.15). IR ν_{max}^{Nujol} cm^{-1}: 3600, 3500, 1720, 1690, 1600, 1590, 1280.

Compound (IV)——The fraction Nos. 436～526 gave a crystalline mass on standing for a few days in acetone. This crystalline mass was recrystallized from acetone many times to give needles, m.p. 175～179° (total 30 mg.). When this material was recrystallized from MeOH+H_2O, the melting point showed 192～197°. Color reactions: Liebermann-Burchard reaction (reddish violet→green), $SbCl_3$ (green). *Anal.* Calcd. for $C_{28}H_{36}O_7$: C, 69.40; H, 7.48, and $C_{28}H_{38}O_7$: C, 69.11: H, 7.87. Found: C, 68.87; H, 7.39. UV λ_{max}^{EtOH} mμ (log ε): 229 (4.19), 273 (3.10), IR ν_{max}^{Nujol} cm^{-1}: 3300, 3200, 1725, 1685, 1600, 1590, 1280, 710.

Reaction of Metaplexigenin (II) with Semicarbazide——To 50 mg. of metaplexigenin (II) dissolved in 2 ml. of EtOH, a solution of 50 mg. of semicarbazide, 60 mg. NaOAc·$3H_2O$ and 0.2 ml. of H_2O was added. After allowing the solution to stand on a boiling water bath for 2 hr., H_2O was added. A crystalline material separated as fine needles, m.p. 262～267°, 41.3 mg. This compound was confirmed as metaplexigenin (II) by mixed melting point.

Metaplexigenin Monooxime (VI)——To 40 mg. of metaplexigenin (II) dissolved in 2 ml. of MeOH, a solution of 100 mg. of NH_2OH·HCl, 300 mg. of NaOAc·$3H_2O$, and 0.2 ml. of H_2O was added. After allowing the solution to stand on a boiling water bath for 3 hr., H_2O was added. Crystals separated out.

Recrystallization of this product from EtOAc afforded 15 mg. of Ⅵ, m.p. 272° (decomp.). *Anal.* Calcd. for $C_{23}H_{35}O_7N$: C, 63.14; H, 8.06; N, 3.20. Found : C, 63.31; H, 8.04; N, 2.99. IR ν_{max}^{Nujol} cm^{-1} : 1745, 1730, 1650, 1240.

Metaplexigenin Monoacetate (VII)——50 mg. of Ⅱ was dissolved in 1 ml. of pyridine and 0.5 ml. of Ac_2O was added. The mixture was allowed to stand for 22 hr. at 25°. The mixture was poured into ice and a white powder which formed was collected and washed several times with H_2O. The mother liquors were extracted with $CHCl_3$-ether(1:3). The extract was treated as usual. Evaporation of the solvent gave a minute quantity of crystals. Repeated recrystallization from MeOH-H_2O gave 33 mg. of Ⅶ, m.p. 262~264°. *Anal.* Calcd. for $C_{25}H_{36}O_8$: C, 64.63; H, 7.81. Found : C, 64.68; H, 7.23. IR ν_{max}^{Nujol} cm^{-1} : 1735, 1695, 1267, 1240.

Hydrolysis of II——A solution of 103 mg. of Ⅱ in 4 ml. of 5% methanolic KOH was refluxed for 5 hr. After adding 2 ml. of H_2O, the MeOH was removed under a reduced pressure. The aqueous solution was extracted successively with small amounts of ether, and the solvent evaporated. After recrystallization from EtOAc+H_2O, it gave 42 mg. of deacylmetaplexigenin (Ⅷ), m.p. 223°. The aqueous layer was acidified with H_3PO_4 and extracted with ether. After the removal of the solvent, 9 mg. of acidic, oily substance remained. The identification of the acid fraction was carried out as follows; paper partition chromatography, solvent system : BuOH/1.5*N* NH_3, paper : Toyo Roshi No. 50 : Acid from metaplexigenin (Rf 0.09), acetic acid (Rf 0.09).

Deacylmetaplexigenin (VIII)——Recrystallization from EtOAc+H_2O afforded Ⅷ, m.p. 217~223°. Color test : $SbCl_3$ (yellowish red→yellowish green), Liebermann-Burchard reaction (pink→yellowish red→yellowish green). *Anal.* Calcd. for $C_{21}H_{32}O_6$: C, 66.30; H, 8.48. Found : C, 66.32; H, 8.41. IR ν_{max}^{Nujol} cm^{-1} : 3500, 3400, 1714, 1690.

Deacylmetaplexigenin Monooxime (IX)——A solution of 37 mg. Ⅷ, 100 mg. of NH_2OHHCl, 300 mg. of $NaOAc \cdot 3H_2O$, in 2 ml. MeOH and 0.2 ml. of H_2O was treated as described for Ⅱ, and crystallized from MeOH+H_2O. 22 mg. of Ⅸ was obtained. *Anal.* Calcd. for $C_{21}H_{33}O_6N$: C, 63.77; H, 8.41; N, 3.54. Found: C, 63.81; H, 8.34; N, 3.74. IR ν_{max}^{Nujol} cm^{-1} : 3350~3450 (broad), 3100, 1640.

Metaplexigenin Monoacetate (VII) from Deacylmetaplexigenin (VIII)——30 mg. of Ⅷ was acetylated as described for Ⅱ, and the product was crystallized from MeOH+H_2O to give 15 mg. of fine prisms, m.p. 255~261°, which were confirmed as Ⅶ by melting point and IR spectra.

Estimation of $Pb(OAc)_4$ Consumption of Sarcostin (I), Metaplexigenin (II) and Deacylmetaplexigenin (VIII)——To a solution of 0.05 mmol. of I, Ⅱ, and Ⅷ dissolved in dioxane (5 ml.), 10 ml. of 1/25*N* $Pb(OAc)_4$ in AcOH was added and the mixture was allowed to stand at room temperature (15~20°) 2 ml. of each mixture was titrated by iodometry. A blank was prepared and titrated similarly. The results are shown in Table Ⅳ.

Table Ⅳ.

Substance	$Pb(OAc)_4$ moles (time : hr.)		
Sarcostin (I)	1.64 (1.5)	2.04 (2.5)	3.03 (125)
Metaplexigenin (Ⅱ)	0.84 (1.0)	0 85 (3.7)	0.90 (164)
Deacylmetaplexigenin (Ⅷ)	1.26 (0.7)	1.67 (18)	2.02 (192)

Sodium Borohydride Reduction of Deacylmetaplexigenin (VIII)——To a solution of 18 mg. of Ⅷ in 1 ml. of dioxane a solution of 10 mg. of $NaBH_4$ in 0.3 ml. of dioxane and H_2O were added. After standing for 24 hr., the solution was acidified to pH 1 with 0.5*N* H_2SO_4 and extracted with ether. Evaporation of the solvent gave 8 mg. of crystals, which were recrystallized from acetone to give needles, m.p. 150/251° (double m.p.). *Anal.* Calcd. for $C_{21}H_{34}O_6$: C, 65.94; H, 8.96. Found : C, 65.95; H, 8.87. This substance was shown to be sarcostin (I) by mixed melting point and IR spectra and paper chromatography ($CHCl_3$/formamide). The mother liquors showed two spots, R_{DC}*[6]=0.20, 0.12 by paperchromatography ($CHCl_3$/formamide), but no crystals was obtained except I by partition chromatography (benzene+BuOH/H_2O) over Celite.

Optical Rotatory Dispersion Measurements of Metaplexigenin (II) and Deacylmetaplexigenin (VIII)——ORD curves of Ⅱ, and Ⅷ were measured with Rudolph Photoelectric Spectropolarimeter, the results and conditions used are as follows : Metaplexigenin (Ⅱ) (Fig. 3) : $[\alpha]_{700}$ −35, $[\alpha]_{315}^{min}$ −756°, $[\alpha]_{265}^{max}$ +1147, $[\alpha]_{254.5}^{min}$ +1107, $[\alpha]_{240}$ +1394°, c=0.202, l=0.1 dm., in MeOH. Deacylmetaplexigenin (Fig. 4) : $[\alpha]_{700}$ +47, $[\alpha]_{316}^{min}$ −309, $[\alpha]_{267}^{max}$ +1449, $[\alpha]_{238}^{min}$ +1435, $[\alpha]_{240}$ +1749°. c=0.213, l=0.1 dm., in MeOH.

*[6] $R_{DC}=R_{deacylcynanchogenin}$

The authors express their deep gratitudes to Dr. K. Takeda, Director of Shionogi Research Laboratory, Osaka for ORD measurements. The authors are also indebted to Mr. S. Shimokawa for NMR measurement and to Mrs. T. Toma, Miss A. Maeda for the elemental analysis.

Summary

The roots of *Metaplexis japonica* MAKINO has proved to contain a glycoside mixture. The acidic hydrolysates of the crude glycoside showed the presence of metaplexigenin and two new aglycones. The structure of metaplexigenin was determined.

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34. Satoshi Toki, Reiko Yamasaki, and Teruko Wakiya : Effect of Phenobarbital Derivatives on the Duration of Hexobarbital Hypnosis.

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Recently it was found[1~4] that the activity of drug-metabolizing enzymes in liver microsomes is increased markedly by the administrations of many drugs and carcinogenic polycyclic hydrocarbons, and this phenomenon has attracted the attention of numerous investigators. Some differences were observed[3] between these two kinds of compounds concerning the ability of stimulation on drug metabolism : for example, drugs such as phenobarbital, barbital, orphenadrine, phenylbutazone and aminopyrine activate the metabolism of hexobarbital, aminopyrine, phenylbutazone, zoxazolamine, 3-methyl-4-methylaminoazobenzene and benzo[*a*]pyrene, but polycyclic hydrocarbons such as benzo[*a*]pyrene and 3-methylcholanthrene stimulate the activity of enzymes which metabolize zoxazolamine, 3-methyl-4-methylaminoazobenzene and benzo[*a*]pyrene. Among the above stimulators, phenobarbital exhibited the most potent activity to the metabolism of most of drugs.

It was also observed[3] that increases in enzyme activity are paralleled by an accelerated drug metabolism in the intact animal and by a shortened duration of drug action.

In the present investigation, phenobarbital derivatives in which either *m*- or *p*-position of the benzene ring was substituted by nitro, amino, dimethylamino, diethylamino, hydroxy, methoxy, chloro, or fluoro group, were synthesized and used together with phenobarbital as a stimulating agent. In order to study a relationship between the structure of phenobarbital derivatives and the activating effect of these compounds on the drug metabolism, the potentiating activities were compared measuring the hexobarbital sleeping time in rats and the hexobarbital metabolism in rat liver.

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