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A New Diglyceride from Root-Barks of *Morus alba* L.[1]

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A new diglyceride, α,β-dimontanyl glycerol (I) has been isolated from the root-barks of *Morus alba* L. The structure of I has been evidenced by chemical and physical methods.

A bush, *Morus alba* L. (Moraceae) are commonly cultivated as the mulberry-tree in the neighborhood of Ina-gun, Miyagi Prefecture. The barks of the genus *Morus* are well known as an antiphlogistic and a diuretic in Japanese herb medicine. Tanemura[3] has reported that an amorphous raw substance which was obtained from a water extract of the root-barks of *Morus alba* L. intensely exhibited depression of blood pressure in mouse.

The present work described the first isolation of a new diglyceride from the root-barks of *Morus alba* L.[4] in the process of the systematic fractionation. A methanol extract of the thermal dried ground roof-barks which defatted with petroleum ether beforehand, was concentrated to syrup under reduced pressure. The residue was triturated with acetone to give a crystalline mass (10 g). This crystal was repeatedly recrystallized from ethyl acetate to yield colorless fine needles (I), mp 82—82.5° in 0.15% yield.

The above petroleum ethereal extract was saponified with alcoholic potassium followed by chromatography on an alumina column to give β-sitosterol (II), mp 138—139°.

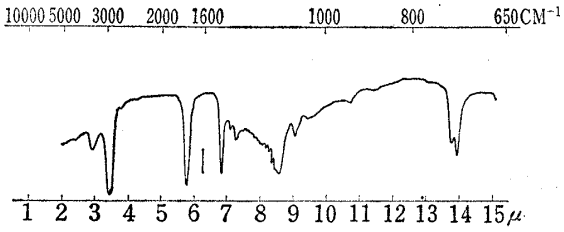

Fig. 1. Infrared Absorption Spectrum of α,β-Dimontanyl Glycerol (I) in KBr

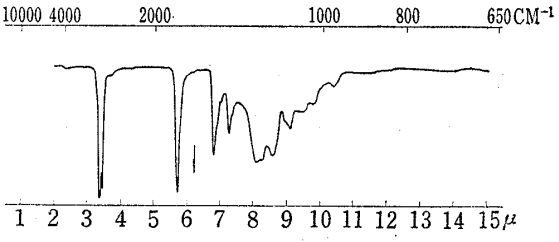

Fig. 2. Infrared Absorption Spectrum of α,β-Dimontanyl-α′-acetyl Glycerol (III) in $CHCl_3$

The elemental analysis of I was consistent with the molecular formula $C_{59}H_{116}O_5$. The infrared (IR) spectrum displayed bands at 3472 cm^{-1} (hydroxyl), 1739 cm^{-1} (ester), 1340—1180, 728.9 and 719.4 cm^{-1} (polymethylene) (Fig. 1). Treatment of I with acetic anhydride in dry pyridine yielded a monoacetate (III) $C_{61}H_{118}O_6$, mp 68.5—69.5° which indicated the absence of a free hydroxyl group in the IR spectrum (Fig. 2). The nuclear magnetic resonance (NMR) spectrum of III (Fig. 3) showed an acetyl signal at 2.04 ppm which was in accord with the result of Kuhn-Roth's acetyl analysis. A diagnostic NMR procedure[5] based on these methine and methylene proton signals at 5.20 ppm and 4.18 ppm indicated that I should be a glyceride. When I and III were subjected to the hydrolysis with 5% alcoholic potassium

1) This work was presented at the Meeting of the Tohoku Branch, the Pharmaceutical Society of Japan, Sendai, October, 1965.
2) Location: *Aobayama, Sendai.*
3) I. Tanemura, *Nippon Yakurigaku Zasshi*, **56**, 704 (1960).
4) The authors are indebted to Dr. Tanemura for supply of *Morus alba* L.
5) J.A. Pople, W.G. Schneider and H.J. Bernstein, "High-resolution Nuclear Magnetic Resonance," McGraw-Hill Book Co. Inc., New York, N. Y., 1959, p. 285.

a sparingly soluble needles (IV), $C_{28}H_{55}O_2K$, mp 240° (decomp.) was separated. The IR spectrum of IV displayed the absorption at 1567 cm^{-1} which was assignable to the carboxylate ion. The filtrate was treated in usual manner followed by extraction with acetone to afford an oily substance. The oil showed a single spot and the *Rf* value was identical with that of an authentic glycerin. Moreover, the reliable proof was given by preparing acrolein.[6]

IV was treated with hydrochloric acid to afford a free acid (V), $C_{28}H_{56}O_2$, mp 83.5—84°. V gave a *p*-bromophenacyl ester (VI), $C_{36}H_{61}O_3Br$, mp 94—95°, and a monomethyl ester (VII), $C_{29}H_{58}O_2$, mp 61.5—63°. V was unambiguously assigned montanic acid by the criteria of molecular ion peak of the methyl ester VII.

On the base of the arguments presented above, the structure of I was presented Ia or Ib. The attempt to prove the hydroxyl position of diglyceride *via* the trityl ether was unsuccessful because of unreactivity of I with trityl chloride. When I was oxidized with chromic acid in acetic acid, a monocarboxylic acid (VIII), $C_{59}H_{114}O_6$, mp 77—80° was obtained. Consequently, the structure of I was represented by the formula Ia.

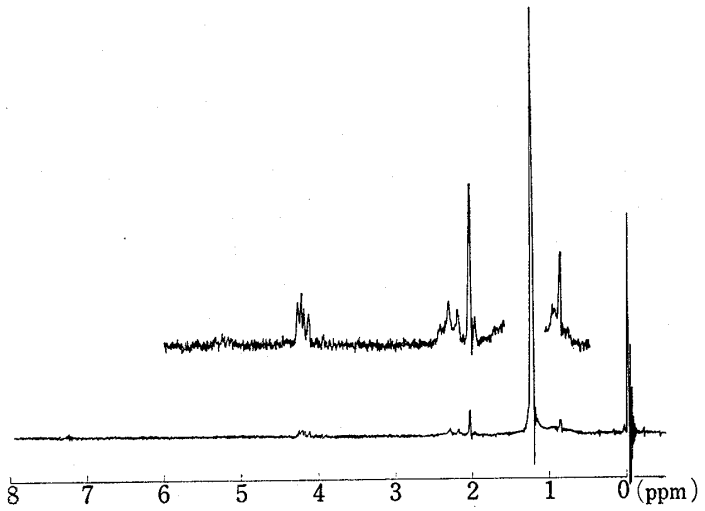

Fig. 3. Nuclear Magnetic Resonance of III in $CDCl_3$

$CH_2OCOC_{27}H_{55}$
$CHOCOC_{27}H_{55}$
CH_2OH
Ia

$CH_2OCOC_{27}H_{55}$
$CHOH$
$CH_2OCOC_{27}H_{55}$
Ib

Experimental[7]

Isolation of Diglyceride (I)——Thermal dried ground root-barks (5.89 kg) of *Morus alba* L. was defatted with petrol ether in a Soxlet apparatus, and then was extracted with MeOH repeatedly. After MeOH was evaporated under reduced pressure, the deposite (108 g) was separated by filtration. The deposite was triturated with acetone to give a crystalline mass (10 g). Recrystallization from AcOEt afforded colorless fine needles, mp 82—82.5° in 0.15% yield (9.1 g). *Anal.* Calcd. for $C_{59}H_{116}O_5$: C, 78.25; H, 12.91. Found: C, 78.01; H, 12.55. IR(KBr) cm^{-1}: 3472 (-OH), 1739 (ester), 1340—1180 (polymethylene), 728.9 and 719.4 (polymethylene).

I Acetate (III)——To a solution of 1.5 g of I in 10 ml of dry pyridine was added 5 ml of acetic anhydride. After heating on a water bath for 5 hr the reaction mixture was evaporated *in vacuo* and added ice water. The deposited crystalline mass was collected and washed with H_2O. Recrystallization from a mixture of $CHCl_3$·MeOH gave monoacetate (III) as colorless needles, mp 68—69.5°. Yield: 1.3 g. *Anal.* Calcd. for $C_{61}H_{118}O_6$: C, 77.31; H, 12.55; Ac, 4.54. Found: C, 77.21; H, 12.72; Ac, 5.75. IR ($CHCl_3$) cm^{-1}: 1747 (ester). NMR ($CDCl_3$) ppm: 0.90 (CH_3-), 1.24 (methylene protons), 2.04 (CH_3CO), 2.30 (t, J=6.6 Hz) (-$COCH_2$-), 4.18 (m) (-$\dot{C}H$-$C\underline{H}_2$-O-), 5.20 (-$\dot{C}\underline{H}$-CH_2-O-).

β-Sitosterol (II)——The petrol ethereal extract was evaporated to dryness *in vacuo*. The residue was hydrolyzed with 5% alcoholic potassium on a water bath for 3 hr. After addition of water, the reaction

6) F. Feigl, "Spot Test in Organic Analysis," Fifth ed., Elsevier Publishing Co., New York, N. Y., 1954, p. 387.

7) All melting points are uncorrected and were taken on a Yanagimoto melting point apparatus. Infrared absorption spectra were recorded on a Hitachi EPI. Nuclear magnetic resonance spectrum was obtained on a Hitachi H-60 spectrometer. Chemical shifts were recorded as δ values (ppm) with TMS internal standard.

mixture was extracted with ether three times. The combined ethereal layer was washed with water and evaporated to afford 4.7 g of an oily residue. The residue was chromatographed on an Al_2O_3 column (20 × 400 mm), the column was eluted with ether and fractionated into 50 ml portions.

Fractions 3 and 4 were pooled and evaporated to give a crystalline residue. Recrystallization from MeOH afforded colorless plates, mp 138—139° (240 mg) which was identical with an authentic β-sitosterol by direct comparison.

Alkaline Hydrolysis of III to Montanic Acid (V)——A solution of III (1.0 g) in 50 ml of 5% EtOH–KOH was refluxed on a water bath for 2 hr. The reaction mixture was poured into water. A water insoluble crystalline mass was separated by filtration, washed with water, and recrystallized from a mixture of MeOH·$CHCl_3$ to afford colorless needles (IV), mp 240° (decomp.). *Anal.* Calcd. for $C_{28}H_{55}O_2K$: C, 72.65; H, 11.97. Found: C, 71.98; H, 11.76. IR (KBr) cm^{-1}: 1567 (-COO^-). The potassium salt (IV) was warmed with 10% HCl aq. for 10 min and the reaction mixture was extracted with $CHCl_3$. The solvent was removed *in vacuo* to yield a crystalline residue. Recrystallization from AcOH gave colorless needles (V), mp 83.5—84°. *Anal.* Calcd. for $C_{28}H_{56}O_2$: C, 79.18; H, 13.29. Found: C, 78.96, 79.06; H, 13.40, 13.51. IR (KBr) cm^{-1}: 3125 (-CO<u>O</u>H), 1709 (-<u>C</u>OOH), 1290—1180 (polymethylene), 728.9 (polymethylene), 718.9 (polymethylene).

Alkaline Hydrolysis of I to IV——1.0 g of I was hydrolyzed with 30 ml of 5% EtOH–KOH. The reaction mixture was worked up as described above. Montanic acid (IV) was obtained as colorless needles, mp 80—82°.

Identification of Glycerin——The water soluble part of the hydrolyzate mentioned above was neutralized with HCl and concentrated to syrup *in vacuo* under 60°. The residue was mixed with powdered anhydrous Na_2SO_4 and extracted with acetone. The solvent was removed *in vacuo* to afford glycerin which was proved as follows.

a) By Paper Chromatography[8]: Paper chromatography of glycerin was performed on Toyo Roshi No. 51 paper in the solvent systems, an upper layer of *n*-BuOH–AcOH–H_2O (4: 1: 5) and *n*-BuOH–AcOH–H_2O–conc. HCl (20: 5: 25: 1). After development spot was detected by spraying with a benzene solution of lead tetraacetate. *Rf*: 0.43 and 0.50 (glycerin 0.43 and 0.50).

b) By Preparation of Acrolein: The test substance was heated with potassium bisulfate as described in the literature.[6] The generated acrolein was detected by its characteristic odor or the color reaction with dianisidine or with sodium nitroprusside.

Preparation of Derivatives of Montanic Acid (V)——a) Methyl Montanate (VII): To a solution of 560 mg of V in 25 ml of $CHCl_3$ was added diazomethane ethereal solution (prepared from 3 g of N-methyl-N-nitroso-*p*-toluenesulfonamide). After standing for 2 hr at room temp. the solvent was removed. The residue was recrystallized from EtOH to afford colorless needles, mp 61.5—63°. *Anal.* Calcd. for $C_{29}H_{58}O_2$: C, 79.38; H, 13.33. Found: C, 79.15; H, 13.48. Mass Spectrum *m/e*: 438 (M^+). IR(KBr) cm^{-1}: 1745 (ester), 1330—1180 (polymethylene), 729.9 (polymethylene), 719.4 (polymethylene).

b) Montanic Acid *p*-Bromophenacyl Ester (VI): To a solution of 440 mg of V in 5 ml of 0.2N EtOH–NaOH was added EtOH solution of *p*-bromophenacyl bromide (278 mg). After refluxing for 1 hr, the reaction mixture was concentrated to yield crystalline mass. Recrystallization from MeOH gave colorless plates, mp 94—95°. *Anal.* Calcd. for $C_{36}H_{61}O_3Br$: C, 69.53; H, 9.88. Found: C, 69.00; H, 9.54.

Chromic Acid Oxidation of I——To a solution of 500 mg of I in hot AcOH was added CrO_3–AcOH solution (prepared from 200 mg of CrO_3 and 2 drops of conc. H_2SO_4 in 5 ml of 90% AcOH).[9] The reaction mixture was allowed to stand overnight under mechanical stirring. After decomposition of excess CrO_3 with aq. EtOH the deposit was collected by filtration. Recrystallization from EtOH gave colorless needles, mp 77—80°. *Anal.* Calcd. for $C_{59}H_{114}O_6$: C, 77.06; H, 12.49. Found: C, 76.97; H, 12.77. IR(KBr) cm^{-1}: 3125 (-CO<u>O</u>H), 1739 (ester), 1712 (-<u>C</u>OOH), 1310—1170 (polymethylene), 728.9 (polymethylene), 719.4 (polymethylene).

Acknowledgement We wish to thank Mr. Sato, Naka Works of Hitachi Ltd., for measuring the mass spectrum.

8) J.G. Buchanan, C.A. Dekker and A.G. Long, *J. Chem. Soc.*, **1950**, 3162.
9) R. Slack and W.A. Waters, *J. Chem. Soc.*, **1948** 1666.