Nonalkaloidal Constituents of *Buxus microphylla* var. *suffruticosa*

By T. NAKANO, S. TERAO, and K. H. LEE

Investigation of the nonalkaloidal constituents from the leaves and stems of *Buxus microphylla* Sieb. et Zucc. var. *suffruticosa* Makino has led to the isolation of methyl syringate, betulic acid, lupeol, and betulin.

THE MEDICINAL properties (1) of the alkaloidal extracts from buxus plants have long been known. A recent investigation (2) has indicated that the leaves and stems of *Buxus microphylla* Sieb. et Zucc. var. *suffruticosa* Makino, like the leaves of *Buxus sempervirens* L. (3), contain a new type of tetracyclic triterpenoid alkaloids with a cyclopropane ring. From a biogenetic point of view, the authors have been interested in examining the nonalkaloidal constituents of this same plant. Chromatography of the alkali-soluble portion on silica gel and elution with chloroform yielded a phenolic compound (I*a*), $C_{10}H_{12}O_5$, m.p. 106–107°; $\lambda_{max.}$ 3.00 (hydroxyl), 5.90 (ester carbonyl), and 6.20, 10.05, and 13.15 μ (benzenoid absorption); $\lambda_{max.}$ 277 mμ (ϵ 1080) (substituted methyl benzoate); NMR peaks at 2.70 (2H, singlet, aromatic protons), 4.00 (1H, OH), 6.08 (6H, singlet, OCH_3), and 6.10 τ (3H, singlet, $COOCH_3$). Methylation of I*a* with diazomethane led to methyl trimethylgallate (I*b*) (4). I*a* was hydrolyzed by alkali to syringic acid (I*c*) (5). The identity of I*a* was established by synthesizing this compound from gallic acid (5). (Scheme I.)

The chloroform–methanol (1:1) eluate fraction was methylated with ethereal diazomethane, and the resultant methyl ester was rechromatographed on alumina. Elution with ether–benzene (1:1) yielded a compound (II*b*), $C_{31}H_{50}O_3$, m.p. 230°; $[\alpha]_D^{27}$ + 1.1° (c, 3); M/e 470 (molecular ion peak). The difficulty (6) of the alkaline hydrolysis of II*b* suggested that its carbomethoxy group is attached to the quaternary carbon atom and possesses the axial orientation. II*b* exhibited infrared absorption bands at 2.80 (nonassociated hydroxyl), 5.85 (ester carbonyl), and 6.07 and 11.36 μ (terminal methylene); NMR signals at 9.26 (3H), 9.19 (3H), 9.10 (3H), 9.06 (6H) (quaternary C-methyl), 8.31 (3H, vinyl methyl), 6.94 (1H, >CH—OH), 6.34 (3H, $COOCH_3$), and 5.39 and 5.26 τ (2H, terminal methylene). Hydrogenation of II*b* with palladium charcoal in ethanol gave the dihydro derivative (III*a*), oxidation with Jones' reagent (7) the oxo derivative (IV); ozonolysis, formalin and a methyl ketone (V); and lithium aluminum hydride reduction the diol (VI*a*) (betulin). Acetic anhydride–pyridine acetylation of II*b*, III*a*, and VI*a*, furnished the acetates (II*c*), (III*b*), and (VI*b*), respectively. The identity of II*b* was established by direct comparison with an authentic sample of the methyl ester of betulic acid (II*a*), kindly supplied by Dr. A. Chatterjee (8), University of Calcutta, Calcutta, India.

Chromatography of the alkali-insoluble portion on silica gel afforded the compound, $C_{30}H_{50}O$, m.p. 213–215°, and the compound, $C_{30}H_{50}O_2$, m.p. 254–255°. The former was found to be identical with lupeol (VII), whose authentic sample was kindly provided by Dr. A. Sosa (9), C. N. R. S., Gif-sur-Yvette, France, and the latter was identified as betulin (VI*a*).

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Rotations were measured with a Kreis polarimeter 0.01° (Carl Zeiss Co. Ltd.) in 1% chloroform solutions unless otherwise indicated. Ultraviolet absorption spectra were recorded on a Shimazu spectrophotometer SV-50 in 95% ethanol. Infrared spectra were taken in KBr disks on a Hitachi spectrophotometer EPI-500. The NMR spectra were obtained by using a Varian A60 spectrophotometer in deuterated chloroform; chemical shifts are reported in τ values using tetramethylsilane as internal standard. (The authors are indebted to Dr. T. Shingu for these determinations.) Alumina for column chromatography refers to Merck's standardized alumina and silica gel to Mallinckrodt's silicic acid.

Extraction and Isolation of the Nonalkaloidal Constituents.—The dried leaves and stems (68 Kg.) of *B. microphylla* Sieb. et Zucc. var. *suffruticosa* Makino were finely ground and extracted three times with warm methanol. The combined methanolic extracts were evaporated *in vacuo*, and the residue was partitioned between 3% aqueous citric acid and the organic solvents (ether, methylene chloride, and ethyl acetate). The organic extracts were combined and evaporated *in vacuo* to yield a dark green sticky residue. A portion (500 Gm.) of this was dissolved in ether and extracted with 1% aqueous sodium hydroxide, whereby acidic (80 Gm.) and neutral (327 Gm.) fractions were obtained.

Methyl Syringate (Ia).—The acidic fraction (25 Gm.) was chromatographed on silica gel (400 Gm.) and elution with chloroform afforded a crystalline material (7.5 Gm.) which was recrystallized from chloroform–ether to form colorless needles, m.p. 106–107°, undepressed by admixture with the compound (I*a*) synthesized from gallic acid.

Anal.—Calcd. for $C_{10}H_{12}O_5$: C, 56.33; H, 6.15. Found: C, 56.64; H, 6.00.

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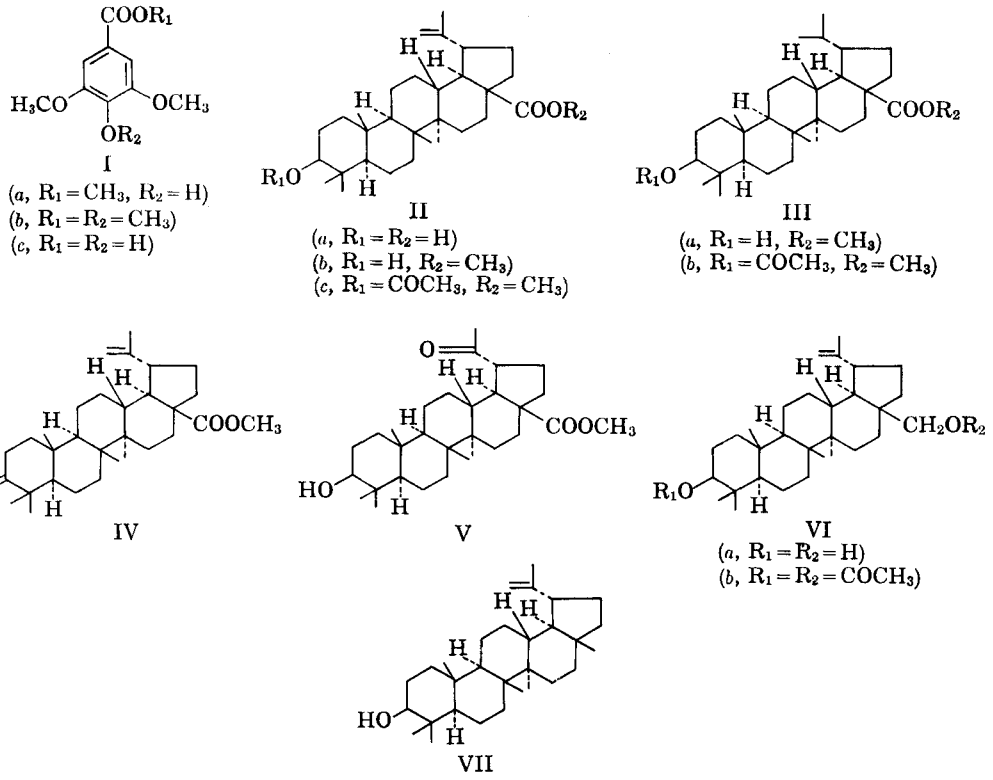

Scheme I

Compound I*a* (100 mg.) in ether was treated with excess ethereal diazomethane. Chromatography of the product on alumina and elution with benzene yielded colorless needles (I*b*), m.p. 80–81°; $\lambda_{max.}$ 5.80 (ester C = O) and 6.26, 9.94, 10.05, 13.00, and 13.10 μ (benzenoid absorption).

Alkaline Hydrolysis of Methyl Syringate.—Compound I*a* (100 mg.) was refluxed with 5% methanolic sodium hydroxide for 15 min. After removal of the solvent *in vacuo*, the residue was diluted with water and extracted with ether. The aqueous layer was acidified with hydrochloric acid and extracted with ether to give colorless crystals (I*c*) (75 mg.), m.p. 202–204°.

Methyl Betulate (IIb).—The chloroform–methanol (1:1) eluate (13 Gm.) from the above silica gel chromatography of the acidic fraction (see for methyl syringate) was methylated in ether solution with excess ethereal diazomethane. Usual working up gave a dark green residue which was taken up in ether and washed successively with 5% sodium carbonate and water. Drying of the ether extract over magnesium sulfate and evaporation yielded the crude ester (10 Gm.). It was then chromatographed on alumina (200 Gm.) and elution with ether–benzene (1:1) afforded a crystalline material (1.6 Gm.) which upon recrystallization from ether–methanol formed colorless slender needles (II*b*), m.p. 230°, undepressed by admixture with methyl betulate. The infrared spectrum of this compound was also identical with that of methyl betulate.

Anal.—Calcd. for $C_{31}H_{50}O_3$: C, 79.10; H, 10.71. Found: C, 78.72; H, 10.92.

Alkaline Hydrolysis of Methyl Betulate.—Compound II*b* (100 mg.) was refluxed with 5% methanolic sodium hydroxide for 2 hr. Isolation of the product in the usual way recovered starting material (90 mg.).

Methyl Acetyl Betulate (IIc).—Compound II*b* (100 mg.) was treated with pyridine (10 ml.) and acetic anhydride (1 ml.), and the mixture left at room temperature for 45 hr. Evaporation of the solution *in vacuo* and isolation of the product in the usual way gave the acetate (II*c*) (96 mg.), m.p. 201–202° (from acetone–methanol); $[\alpha]_D^{28}$ + 15°; $\lambda_{max.}$ 5.78 (C=O) and 6.08 and 11.35 μ (terminal methylene); NMR peaks at 9.16 (9H), 9.08 (3H), 9.04 (3H) (quaternary C-methyl), 8.31 (3H, vinyl methyl), 7.96 (3H, $OCOCH_3$), 6.32 (3H, $COOCH_3$), and 5.37 and 5.25 τ (2H, two doublets, $J < 1$ c.p.s., terminal methylene).

Anal.—Calcd. for $C_{33}H_{52}O_4$: C, 77.29; H, 10.22. Found: C, 77.04; H, 10.50.

Methyl Dihydrobetulate (IIIa).—Compound II*b* (60 mg.) was hydrogenated in 95% ethanol (15 ml.) with prehydrogenated 5% palladium charcoal (30 mg.) at atmospheric pressure and 27°. Usual working up of the product and crystallization from ether–methanol afforded colorless needles (45 mg.), m.p. 244°; $[\alpha]_D^{27}$ − 31°; $\lambda_{max.}$ 2.81 (nonassociated OH) and 5.88 μ (ester C=O); NMR peaks at 9.23 (3H), 9.19 (3H), 9.16 (3H), 9.08 (6H), 9.06 (3H), 9.04 (3H) (quaternary C-methyl), 6.83 (1H, multiplet, >CH—OH) and 6.34 τ (3H, $COOCH_3$).

Anal.—Calcd. for $C_{31}H_{52}O_3$: C, 78.76; H, 11.09 Found: C, 79.09; H, 10.96.

Methyl Acetyl Dihydrobetulate (IIIb).—A mixture of IIIa (43 mg.), pyridine (15 ml.), and acetic anhydride (1 ml.) was allowed to stand at room temperature for 18 hr. The product was chromatographed on alumina and elution with ether gave the acetate (IIIb) (36 mg.), m.p. 236–237° (from ether–methanol); $[\alpha]_D^{25}$ − 8°; $\lambda_{max.}$ 5.78 μ (C=O); NMR peaks at 7.95 (3H, $OCOCH_3$) and 6.36 τ (3H, $COOCH_3$).

Anal.—Calcd. for $C_{33}H_{54}O_4$: C, 76.99; H, 10.57. Found: C, 76.71; H, 10.62.

Methyl Oxobetulate (IV).—To a stirred solution of IIb (60 mg.) in acetone (30 ml.) was added dropwise at room temperature Jones' reagent (0.04 ml.). After 15 min., the resulting green solution was diluted with water, and the product was extracted with ether. The ether extract was washed with water, dried, and evaporated to leave a residue (90 mg.). Chromatography of this on alumina (2 Gm.) in benzene afforded colorless needles (IV) (31 mg.), m.p. 165–166° (from methanol); $\lambda_{max.}$ 5.77 (ester C=O), 5.85 (6-membered ring ketone), and 6.08 and 11.35 μ (terminal methylene); NMR peaks at 9.07 (3H), 9.04 (6H), 8.96 (3H), 8.92 (3H) (quaternary C-methyl), 8.30 (3H, vinyl methyl), 6.32 (3H, $COOCH_3$), and 5.38 and 5.25 τ (2H, two doublets, J < 1 c.p.s., terminal methylene).

Anal.—Calcd. for $C_{31}H_{48}O_3$: C, 79.43; H, 10.32. Found: C, 79.23; H, 10.63.

Ozonolysis of Methyl Betulate.—Compound IIb (150 mg.) in methylene chloride (20 ml.) was ozonized at −75° until the solution became slightly bluish. The excess ozone and methylene chloride were removed *in vacuo*, and the residue was treated with acetic acid (20 ml.) and zinc dust (500 mg.) for 2 hr. After filtration of the zinc dust, the filtrate was steam distilled. The volatile fraction led to the 2,4-dinitrophenylhydrazone derivative, m.p. 157°, undepressed by admixture with formaldehyde 2,4-dinitrophenylhydrazone.

The nonvolatile fraction was made alkaline with ammonia and extracted with ether. Washing of the extract with water, drying, and evaporation left a residue which was chromatographed on alumina (3 Gm.). Elution with ether–benzene (1:9) furnished a methyl ketone (V) (45 mg.) as colorless needles, m.p. 251–252° (from methylene chloride–methanol); $[\alpha]_D^{25}$ − 29°; $\lambda_{max.}$ 2.87 (nonassociated OH), 5.78 (ester C=O), and 5.90 μ (methyl ketone), NMR peaks at 9.24 (3H), 9.17 (3H), 9.11 (3H), 9.04 (3H), 9.02 (3H) (quaternary C-methyl), 7.82 (3H, $COCH_3$), 6.82 (1H, multiplet, >CH—OH) and 6.32 τ (3H, $COOCH_3$).

Anal.—Calcd. for $C_{30}H_{48}O_4$: C, 76.22; H, 10.24. Found: C, 76.33; H, 10.22.

Diol (Betulin) (VIa).—A solution of IIb (100 mg.) in absolute ether was added to a suspension of lithium aluminum hydride (200 mg.) in dry ether. After the addition, the whole was stirred at room temperature for 5 hr. After the excess reagent was destroyed with ethyl acetate, a concentrated aqueous sodium sulfate solution was added to precipitate inorganic salts, followed by the addition of anhydrous magnesium sulfate. Decantation of the ether solution and evaporation yielded a product which was purified by chromatography on alumina. Elution with ether–benzene (1:2) furnished a diol (VIa) (70 mg.) which upon recrystallization from methylene chloride–methanol formed colorless needles, m.p. 254–255°; $[\alpha]_D^{25}$ + 21° (c, 0.5); $\lambda_{max.}$ 2.91 (OH) and 6.09 and 11.34 μ (terminal methylene); NMR peaks at 9.22 (3H), 9.16 (3H), 9.02 (6H), 8.96 (3H) (quaternary C-methyl), 8.30 (3H, vinyl methyl), 6.92 (1H, multiplet, >CH—OH), 6.66, 6.18 (2H, quadruplet centered at 6.42, J = 11 c.p.s., $-CH_2OH$), and 5.39 and 5.31 τ (2H, two doublets, J < 1 c.p.s., terminal methylene).

Betulin Diacetate (VIb).—Compound VIa (45 mg.) was acetylated with acetic anhydride–pyridine (5:1) (6 ml.) at room temperature overnight. The product was crystallized from methylene chloride–methanol to give colorless needles (31 mg.), m.p. 223°; $[\alpha]_D^{25}$ + 37°; $\lambda_{max.}$ 5.77 (O—$COCH_3$) and 6.10 and 11.24 μ (terminal methylene).

Lupeol (VII) and Betulin.—The neutral fraction (45 Gm.) was chromatographed on silica gel (400 Gm.) and elution with benzene–ether (9:1) gave a material (1.95 Gm.) which was recrystallized from ether–methanol to form colorless slender needles (1.5 Gm.), m.p. 213–215°; $[\alpha]_D^{25}$ + 25°; $\lambda_{max.}$ 2.95 (nonassociated OH) and 6.09 and 11.33 μ (terminal methylene); NMR peaks at 9.25 (3H), 9.21 (3H), 9.18 (3H), 9.05 (6H), 8.98 (3H) (quaternary C-methyl), 8.32 (3H, vinyl methyl), 6.83 (1H, multiplet, >CH—OH), and 5.43 and 5.32 τ (2H, two doublets, J < 1 c.p.s., terminal methylene). This compound showed no melting point depression on admixture, and its infrared spectrum was identical with that of lupeol.

Elution with methanol–ether (1:9) afforded a diol (10 Gm.) which was acetylated with acetic anhydride (25 ml.) and pyridine (50 ml.) at room temperature overnight. The product was chromatographed on alumina (300 Gm.), and the benzene eluate was evaporated to give colorless slender needles (from ether–methanol), m.p. 223°; $[\alpha]_D^{27}$ + 36°. The melting point of this compound was undepressed by admixture of betulin diacetate (VIb), and also their infrared spectra were identical.

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