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SHORT COMMUNICATION

TRITERPENE CONSTITUENTS OF *TABERNAEMONTANA LAURIFOLIA* AND *HAPLOPHYTON CIMICIDUM*

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Abstract—The triterpene bauerenyl acetate was isolated directly by crystallization from the non-polar neutral extract of the bark of *Tabernaemontana laurifolia* D.C. A similar extract of *Haplophyton cimicidum* (whole plant) yielded, after chromatography, the triterpenes erythrodiol monostearate, bauerenol and betulin, as well as the steroid β-sitosterol.

IN THE course of investigating the alkaloidal constituents of various plants of the family *Apocynaceae*, we reserved some of our neutral residues for an examination of their triterpene constituents. We now report the isolation and identification of some neutral constituents of *Tabernaemontana laurifolia* D.C. and of *Haplophyton cimicidum*.

The powdered bark of *T. laurifolia*, after extraction of the alkaloids present[1] with aqueous tartaric acid, was treated with methylene chloride to remove the triterpene constituents. The hexane-soluble portion of methylene chloride extract crystallized directly to give a single compound, $C_{32}H_{52}O_2$, m.p. 282–283°. This substance was shown to be bauerenyl acetate (I) by direct comparison with authentic material as well as by hydrolysis to bauerenol (II). An attempt to isolate other triterpenes by chromatography of the neutral residues of the *T. laurifolia* extract was not successful.

H. cimicidum (whole plant) was extracted with benzene, and the alkaloids present[2] were removed by extraction with aqueous mineral acid. The hexane-soluble portion of the neutral benzene extract yielded, after careful chromatography, four crystalline products shown to be the triterpenes bauerenol (II), betulin (III) and erythrodiol monostearate (IV), and the common plant sterol β-sitosterol (V).

Bauerenol has been reported previously only from a small number of plants, i.e., *Achronychia baueri* (Rutaceae),[3] *Gelonium multiflorum* (Euphorbiaceae)[4] and *Kopsia longiflora* (Apocynaceae).[3, 5] Erythrodiol monostearate has been reported previously only as a constituent of *Erythroxylon novogranatense* (Erythroxylaceae).[6]

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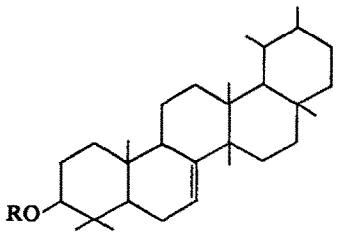
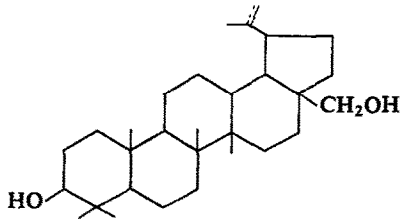
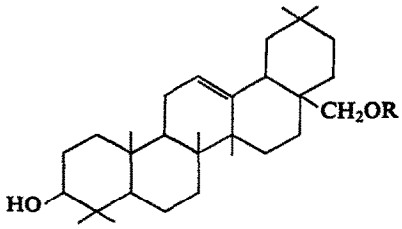


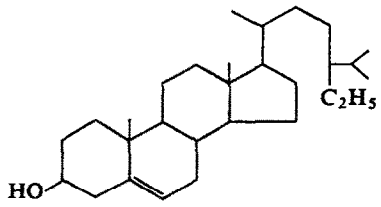
(I) R=$COCH_3$
(II) R=H



(III)



(IV) R=CO—$C_{17}H_{35}$



(V)

EXPERIMENTAL

Melting points are uncorrected. Optical rotations were determined in chloroform solution. Elemental analyses were carried out by Dr. A. Bernhardt, Mulheim, Germany, and by Midwest Microlab, Indianapolis, Indiana.

Tabernaemontana laurifolia: *bauerenyl acetate.* The finely ground bark was extracted repeatedly with hot 1% aqueous tartaric acid, followed by hot water, and then air-dried. A portion of the resulting powder (6·8 kg) was percolated with methylene chloride (85 l.). Removal of the solvent gave a brown gum (344 g) which was triturated with hexane (4 l.) until it became powdery. The solid precipitate (47 g) was removed by filtration and the filtrate was concentrated, when crystals separated. Recrystallization from benzene gave colorless plates (6·0 g) of bauerenyl acetate, m.p. 282–283° (lit.[3] 293–294°); $[\alpha]_D - 3$ (lit.[3] −4). (Found: C, 81·88; H, 11·05. Calc. for $C_{32}H_{52}O_2$: C, 81·99; H, 11·18%). Hydrolysis of bauerenyl acetate (500 mg) with methanolic KOH (10%; 20 ml) and benzene (25 ml) for 6 hr on the steam bath afforded, after crystallization from benzene–methanol, bauerenol, m.p. 198° (lit.[3] 204–205°); $[\alpha]_D - 21$ (lit.[3] −30). (Found: C, 84·27; H, 11·89. Calc. for $C_{30}H_{50}O$: C, 84·44; H, 11·81%.) The identity of the bauerenyl acetate was further confirmed by comparison (i.r. and NMR) with an authentic sample.

Haplophyton cimicidum. The material investigated was an aliquot portion of a benzene extract of the whole plant, from which the alkaloids had been removed by extraction with aqueous sulfuric acid. The benzene was evaporated to dryness, and the hexane-soluble portion of the residue was chromatographed on a large column of neutral alumina (grade II). After a forerun of waxy material eluted by hexane, benzene and benzene–ether (4:1) eluted crystalline fractions. The combined crystals (15 g) were dissolved in chloroform and adsorbed over neutral alumina (grade II) (100 g). The dried material was placed on the top of a column of fresh alumina (300 g) and eluted with benzene–hexane (1:1), benzene and benzene–ether (4:1).

Erythrodiol monostearate. The earlier fractions of the benzene–hexane eluate gave erythrodiol monostearate crystallizing from acetone as colorless shining flakes, m.p. 121–123°, $[\alpha]_D + 72·5°$. (Found: C, 81·22; H, 11·94. Calc. for $C_{48}H_{84}O_3$: C, 81·29; H, 11·94%.) Lit.[6] m.p. 125°, $[\alpha]_D + 49·9°$.

Erythrodiol. Erythrodiol monostearate (500 mg) was hydrolyzed with ethanolic KOH solution (10%; 25 ml) under reflux for 3 hr. After the usual work-up, the product yielded erythrodiol, crystallizing from benzene as a colorless microcrystalline solid, m.p. 231–233°, $[\alpha]_D + 74·4°$, identical (i.r.) with an authentic sample. (Found: C, 81·51; H, 11·34. Calc. for $C_{30}H_{50}O_2$: C, 81·39; H, 11·38%.) Lit.[6] m.p. 232°, $[\alpha]_D + 75·4°$.

Erythrodiol diacetate. Erythrodiol (250 mg) was acetylated with acetic anhydride and pyridine (3 ml each) on the steam bath for 3 hr. Crystallization of the product from methanol furnished colorless needles of erythrodiol diacetate, m.p. 183–184°, $[\alpha]_D + 65·5°$, identical (i.r.) with an authentic specimen. (Found: C, 77·53; H, 10·53. Calc. for $C_{34}H_{54}O_4$: C, 77·52; H, 10·33%.) Lit.[6] m.p. 187°, $[\alpha]_D + 59·4°$.

Bauerenyl acetate. The latter fractions of the benzene–hexane eluate afforded a solid, m.p. 185–190°. 500 mg of the solid was acetylated in the usual manner with acetic anhydride and pyridine (5 ml each). A crude acetate, m.p. 270–275° crystallized out of the reaction medium. Crystallization from chloroform–methanol

furnished bauerenyl acetate as shining flakes, m.p. 287°, $[\alpha]_D -4°$, identical (i.r.) with an authentic sample. (Found: C, 81·44; H, 11·14. Calc. for $C_{32}H_{52}O_2$: C, 81·99; H, 11·18%.) Lit.[3] m.p. 293–294°, $[\alpha]_D -4°$.

Bauerenol. Bauerenyl acetate (100 mg) was hydrolyzed under reflux with ethanolic KOH (10%; 20 ml) for 3 hr. The product was crystallized from methanol to furnish bauerenol, m.p. 204–205°, $[\alpha]_D -29·2°$, identical (i.r.) with an authentic sample. Lit.[3] m.p. 207–208°, $[\alpha]_D -30°$.

β-Sitosterol benzoate. The benzene eluate on evaporation left a solid which gave in the Liebermann–Burchard test a transient pink color changing to blue and finally green. The sterol (500 mg) was benzoylated with benzoyl chloride (2 ml) and pyridine (3 ml) at room temperature overnight. Crystallization of the product from benzene–ethanol yielded colorless flakes of β-sitosteryl benzoate, m.p. 145–147°, $[\alpha]_D -12·9°$, identical (i.r.) with an authentic sample.

β-Sitosterol. Hydrolysis of β-sitosteryl benzoate (200 mg) with ethanolic KOH (5%; 25 ml) under reflux for 3 hr furnished β-sitosterol crystallizing as shining leaflets from methanol, m.p. and m.m.p. 136–137°.

Betulin diacetate. The benzene–ether (4:1) eluate gave a residue crystallizing from methanol as a colorless solid, m.p. 205–230°. The solid (150 mg) was acetylated in the usual manner with acetic anhydride and pyridine (3 ml each) and the product was purified by chromatography in hexane solution over neutral alumina (grade II). Elution of the column with benzene–hexane (1:9) and crystallization of the residue from methanol furnished colorless needles of betulin diacetate, m.p. 218–219°, $[\alpha]_D +22°$, identical (i.r.) with an authentic sample. (Found: C, 77·36; H, 10·44. Calc. for $C_{34}H_{54}O_4$: C, 77·52; H, 10·33%.) Lit.[7] m.p. 223°, $[\alpha]_D +22°$.

Betulin. Betulin diacetate (70 mg) on hydrolysis with ethanolic KOH (5%; 10 ml) furnished microcrystalline needles from methanol, m.p. 253–254°, $[\alpha]_D +20·3°$, identical (i.r.) with an authentic sample. Lit.[7] m.p. 261°, $[\alpha]_D +20°$.

Acknowledgement—The authors are grateful to Dr. F. N. Lahey for the generous gift of a sample of authentic bauerenyl acetate.

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