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SHORT COMMUNICATIONS

AN EXAMINATION OF THE RUBIACEAE OF HONG KONG—II.*

THE OCCURRENCE OF TRITERPENOIDS AND STEROIDS FROM *HEDYOTIS ACUTANGULA* CHAMP.

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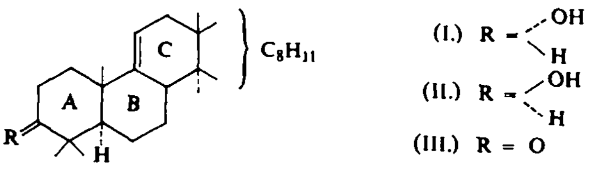
Abstract—The leaves of *Hedyotis acutangula* Champ. yield the triterpenoids, arborinone and isoarborinol; and the sterols, stigmasterol and γ-sitosterol. Arborinol and isoarborinol, members of a new type of triterpenoid, have only been isolated recently from *Glycosmis arborea*.[1] Arborinone, which has been obtained as oxidation product of both arborinol and isoarborinol,[2] has not been previously isolated from natural sources.

RESULTS AND DISCUSSION

Hedyotis acutangula Champ. is a plant, 2–3 ft tall with erect and very acutely quadrangular branches. Its leaves and fruits are sessile; the former being 2–3 in. long and the latter are small and numerous.

Chromatography on alumina of the light petroleum extract of the dry leaves first yielded [redacted] mixture was obtained from which stigmasterol and γ-sitosterol were separated as their 3,5-dinitrobenzoates.[3]

We report here the first isolation of arborinone from a natural source. Arborinol and isoarborinol were first isolated from *Glycosmis arborea* (Rutaceae) by Pakrashi and Roy,[1] and were proved to be epimeric triterpene alcohols by Vorbrüggen, Pakrashi and Djerassi,[2] who oxidized both arborinol and isoarborinol to arborinone, which on reduction yielded mainly isoarborinol with a trace of arborinol. They established the partial structures of arborinol, isoarborinol and arborinone as (I), (II) and (III) respectively, with rings D and E similar to those of either ursane, lupane, or hopane.



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[*] The occurrence of triterpenoids and steroids in some plants of the Rubiaceae family of Hong Kong, *Aust. J. Chem.* **17**, 493 (1964), is regarded as Part I of the series.

[1] S. C. Pakrashi and S. K. Roy, *J. Sci. Ind. Res.* (*India*), **20B**, 186 (1961).

[2] H. Vorbrüggen, S. C. Pakrashi and C. Djerassi, *Ann.* **668**, 57 (1963).

[3] A. E. Atherinos, I. E. El-Kholy and G. Soliman, *J. Chem. Soc.* 1700 (1962).

In our work arborinone was subjected to Wolff-Kishner reduction to give arborene. Isoarborinyl acetate and benzoate were prepared, and the former was hydrolysed to the original isoarborinol. Isoarborinol was oxidized to arborinone, and subjected to retropinacol rearrangement. Both arborinone and isoarborinol isolated by us showed i.r. spectra and mixed m.p. identity with authentic samples kindly supplied by Professor C. Djerassi.

EXPERIMENTAL

Analyses were by the Microanalytical Laboratory, University of Singapore. Melting points were determined on a Kofler block. The alumina used for chromatography was B.D.H. analysis grade. Light petroleum had b.p. 60–80°. Infrared spectra were taken on a Perkin Elmer Infracord (model 137) spectrophotometer. Ultraviolet spectra were taken on a Unicam, S.P. 500 spectrophotometer. Mixed melting points with authentic samples were determined for arborinone (both naturally occurring and from the oxidation of isoarborinol), isoarborinol, stigmasterol, γ-sitosterol and γ-sitosteryl acetate. No depressions were observed. The i.r. spectra of these compounds were found to be identical with those of the authentic samples.

Extraction and Isolation of Products

Air dried leaves (5 kg) were extracted twice with light petroleum (20 l.) at room temperature for 10 days. The extract was concentrated to 200 ml. and chromatographed on alumina (600 g). Elution with light petroleum yielded a very small amount of colourless plates, m.p. 111–112°, which soon turned yellow. This compound gave a negative Liebermann–Burchardt test and a negative alkaloid test. It was not further investigated. Elution with light petroleum: benzene (4:1) yielded plates of a compound (0·15 g), m.p. 180–190°, which on recrystallization from acetone gave rectangular prisms, m.p. 217–220°, which in the Liebermann–Burchardt test gave a purple→blue→green colour. Test with tetranitromethane gave a pale yellow colour. Elution with light petroleum:benzene (1:4) yielded needles of a second compound (0·5 g), m.p. 305–306°. (Liebermann–Burchardt test: purple→blue→green colour; tetranitromethane test pale yellow colour.) Further elution with pure benzene gave a steroid mixture (0·5 g) m.p. 138–142°.

Arborinone

Crystals obtained from fractions eluted with light petroleum: benzene (4:1) were recrystallized three times from acetone as plates of arborinone, m.p. 217–220°, $[\alpha]_D + 30{\cdot}0°$ (*c*, 0·720 in $CHCl_3$) (Djerassi *et al.*[2] reported m.p. 214–214·5°, $[\alpha]_D + 28{\cdot}8°$) (Found: C, 85·2; H, 11·4. Calc. for $C_{30}H_{48}O$: C, 84·8; H, 11·4%). $\gamma_{max.}$ 1700 cm^{-1} (C = O). Arborinone (20 mg) was subjected to Wolff–Kishner reduction in diethylene glycol and yielded plates of arborene (4 mg) m.p. 251° (reported[2] 247–247·5°).

Isoarborinol and Derivatives

Crystals from fractions eluted with light petroleum: benzene (1:4) were recrystallized from benzene to give needles of isoarborinol, m.p. 305–306°, $[\alpha]_D + 40{\cdot}0°$ (*c*, 0·46, in $CHCl_3$) (Djerassi *et al.*[2] reported m.p. 295–296°, $[\alpha]_D + 47{\cdot}0°$) (Found: C, 83·9; H, 12·0. Calc. for $C_{30}H_{50}O$: C, 84·4; H, 11·8%). $\gamma_{max.}$ 3650 cm^{-1} (OH). Acetylation gave isoarborinyl acetate which was recrystallized from chloroform–ethanol as hexagonal prisms, m.p. 296°, $[\alpha]_D + 54{\cdot}1°$ (*c*, 0·762 in $CHCl_3$) (reported[2] m.p. 287–288°, $[\alpha]_D + 56{\cdot}0°$) (Found: C, 82·3;

H, 11·4. Calc. for $C_{32}H_{52}O_2$: C, 82·0; H, 11·2%). $\gamma_{max.}$ 1730, 1240 cm^{-1} (OAc). The compound was hydrolysed and gave the original alcohol, m.p. 298–300°. *Isoarborinyl benzoate* was prepared and purified by chromatography on alumina. It was recrystallized from chloroform–methanol as needles, m.p. 291–292°, $[\alpha]_D + 45·2°$ (*c*, 0·354 in $CHCl_3$) (Found: C, 83·3; H, 10·7. Calc. for $C_{37}H_{54}O_2$; C, 83·7; H, 10·3%). $\gamma_{max.}$ 1715, 1280, 1115 cm^{-1} (PhCOO).

Reactions of Isoarborinol

(*a*) *Oxidation of isoarborinol.* Isoarborinol (0·15 g) was dissolved in stabilized acetone and oxidized with chromic acid at room temperature. The product was recrystallized from acetone to give plates of arborinone (0·1 g) ,m.p. 218–219°, $[\alpha]_D + 30·7°$ (*c*, 0·446 in $CHCl_3$) (Found: C, 85·0; H, 11·4. Calc. for $C_{30}H_{48}O$: C, 84·8; H, 11·4%).

(*b*) *Retropinacol rearrangement.* Isoarborinol (0·15 g) was dissolved in sodium-dried light petroleum (500 ml.) and excess phosphorus pentachloride (0·5 g) was added and the mixture was shaken for 1 hr. The product, after chromatography, yielded needles of a hydrocarbon (0·12 g) m.p. 184–189° (reported[2] m.p. 175–182°).

Stigmasterol

The sterol mixture (0·75 g) was dissolved in pyridine (10 ml), and excess 3:5-dinitrobenzoyl chloride (3 g) was added, then the mixture was heated under reflux for 4 hr. The product was poured into cold water and filtered. The residue was digested with ethanol and recrystallized from chloroform–ethanol, benzene–ethanol and then ethyl acetate, The product which was obtained as yellowish plates (0·13 g.) had m.p. 223–225° It was dissolved in chloroform–ethanol (40 ml) containing potassium hydroxide (0·5 g) and heated under reflux for 2 hr. The mixture was then poured into water, and the product collected and recrystallized from ethanol to give plates (50 mg), m.p. 163–165°, $[\alpha]_D\ -48°$ (*c*, 0·48 in $CHCl_3$). The acetate was prepared and crystallized from ethanol as plates, m.p. 140–142°.

γ-Sitosterol

The first mother liquor left after separation of stigmasteryl 3:5-dinitrobenzoate was evaporated to dryness and boiled under reflux in ethanolic potassium hydroxide for 2 hr. The product was recrystallized from ethanol to give plates (0·2 g), m.p. 142–145°. This crude product was purified by chromatography on alumina (15 g) and yielded γ-sitosterol, m.p. 144–148°, $[\alpha]_D\ -43°$ (*c*, 1·06 in $CHCl_3$). Acetylation gave γ-sitosteryl acetate, m.p. 138–140°, $[\alpha]_D\ -43°$ (*c*, 0·34 in $CHCl_3$).

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