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SHORT COMMUNICATION

TRITERPENES FROM *THUIDIUM TAMARISCIFOLIUM**

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Abstract—Nine triterpenes and sterols have been isolated from *Thuidium tamariscifolium*. A number of aliphatic hydrocarbons and fatty acids were also detected.

FROM the light petroleum extract of the moss *Thuidium tamariscifolium* (Neck.) Lindb. (= *Thuidium tamariscinum* B. e.) the following triterpenes and sterols were isolated and identified: 22(29)-Hopene,[2] 7-fernene,[2c, 3] 9(11)-fernene,[2b, 3, 4] 24-methylenecycloartanol,[5] lupeol, ursolic acid, campesterol (identified by GLC), stigmasterol (identified by GLC) and β-sitosterol. Moreover, aliphatic hydrocarbons from C_{22} to C_{33} were also found in the plant and, after saponification of the extract, fatty acids from C_{14} to C_{22}.

In our first communication on this subject,[1] we remarked that hopane hydrocarbons seem to be characteristic of primitive plants; their occurrence in ferns and mosses also points to a possible phylogenetic connexion between bryophytes and pteridophytes.[6, 7] Further studies, now in progress, seem to support this hypothesis.

EXPERIMENTAL

M.ps were determined with a Kofler apparatus and are uncorrected. Infrared spectra were registered on nujol mulls with a Perkin–Elmer Infracord, Mod. 137 spectrophotometer. GLC analyses were performed with the following instruments: Perkin–Elmer F 20, for triterpenes and sterol trimethylsilyl ethers (column, 3 per cent SE 52 silicone rubber on Chromosorb W 80–100 mesh, temp. 240°, injection block temp. 260°, carrier gas N_2, flow rate 50 ml/min); Carlo Erba Fractovap, Mod. G.V., for aliphatic hydrocarbons (column, 1 per cent neopentyl glycol succinate on Chromosorb W 80–100 mesh, programmed temp. from 190° to 230°, increase 1·5°/min, injection block temp. 240°, carrier gas N_2, flow rate from 39 to 32 ml/min); Perkin–Elmer 800, for fatty acid methyl esters (column, 20 per cent polyethylene glycol succinate on acid-washed Chromosorb W 80–100 mesh, temp. 200°, injection block temp. 290°, carrier gas N_2, flow rate 40 ml/min). Specific rotations were measured with a Perkin–Elmer, Mod. 141 photoelectric polarimeter, at 25°. Light petroleum refers to the fraction of boiling range 30–60°. Identities of products were determined by comparison of i.r. spectra and GLC retention times.

* "Triterpenes from Mosses-II". For Part I see Ref. 1.

[1] Part I, A. MARSILI and I. MORELLI, *Phytochem.* 7, 1705 (1968).
[2] (a) W. J. DUNSTAN, H. FAZAKERLEY, T. G. HALSALL and E. R. H. JONES, *Croat. Chem. Acta* **29**, 173 (1957); (b) H. AGETA, K. IWATA and K. YONEZAWA, *Chem. Pharm. Bull. Japan* **11**, 408 (1963); (c) H. AGETA, K. IWATA and S. NATORI, *Tetrahedron Letters* 3413 (1964).
[3] H. AGETA and K. IWATA, *Tetrahedron Letters* 6069 (1966).
[4] H. AGETA, K. IWATA and S. NATORI, *Tetrahedron Letters* 1447 (1963).
[5] Triterpenoids of the cyclolanostane group were also found in ferns. See, e.g., G. BERTI, F. BOTTARI, B. MACCHIA, A. MARSILI, G. OURISSON and H. PIOTROWSKA, *Bull. soc. chim. Fr.* 2359 (1964).
[6] See G. M. SMITH, *Cryptogamic Botany*, Vol. II, pp. 131–134, McGraw-Hill, New York (1955).
[7] G. HASKALL [*Bryologist* **52**, 49 (1949)] holds that bryophytes descend from pteridophytes.

Extraction

The dried plant (3 kg), collected on April 1968 in a wood of *Castanea sativa* Mill., was extracted in a Soxhlet with light petroleum (8 l.) for 45 hr. On cooling a ppt (0·25 g) separated out; this was combined with the ppt (0·27 g) formed when the filtrate was evaporated to 2 l. Crystallization of the solid from EtOH afforded ursolic acid.[8] Further concentration of the extract to 250 ml gave waxes (3·1 g), m.p. 75–81°, after crystallization from benzene–MeOH; λ_{CO} 5·80 μ. The remaining soln. was evaporated to dryness to give a brown residue (31 g) which was refluxed with 10 per cent NaOH–EtOH (200 ml) for 4 hr. After this time the solvent was removed by distillation and the residue, dried at 90°, was extracted in a Soxhlet with light petroleum (300 ml) for 100 hr. The extract, on evaporation, afforded 6 g of unsaponifiable material. The unextracted solid was taken up in water and the soln. was acidified with conc. HCl and extracted with Et_2O. Evaporation of the Et_2O afforded 15 g of fatty acids, 1 g of which was converted into the corresponding methyl esters with CH_2N_2.

Unsaponifiable Material

A part of the unsaponifiable fraction (5 g) was dissolved in light petroleum and chromatographed over neutral alumina (Fluka, grade I, 200 g, 2 × 65 cm column). Light petroleum eluted hydrocarbons (aliphatic and triterpenoidic); no residue was obtained with benzene; Et_2O eluted mixtures of alcohols. The combined hydrocarbon fractions (0·4 g) were re-chromatographed over silica gel (Woelm, grade I, 120 g, 2 × 52 cm column; 20-ml fractions) impregnated with 15 per cent of $AgNO_3$. Light petroleum eluted aliphatic hydrocarbons (0·12 g), 9(11)-fernene (5 mg) and 7-fernene (35 mg); benzene eluted 22(29)-hopene (0·12 g). The alcoholic fractions, analysed by GLC (as trimethylsilyl ethers) contained at least eight components (mainly campesterol, stigmasterol, β-sitosterol and 24-methylenecycloartanol). A fraction (1·1 g) showing in the i.r. spectrum a band at 11·25 μ ($>C=CH_2$) was benzoylated; the resulting mixture of benzoates was chromatographed over silica gel–$AgNO_3$ (110 g, 2 × 50 cm column; 20-ml fractions), using as eluant light petroleum containing increasing amounts of benzene. The fractions obtained with light petroleum–benzene (1:1) were combined and the residue (0·12 g) crystallized from light petroleum. Lupeol benzoate[8] (30 mg) separated out. The mother liquor, after evaporation, left a residue which was crystallized from acetone–MeOH; 24-methylenecycloartanol benzoate (20 mg) separated out. Another alcoholic fraction (0·5 g) containing campesterol (30 per cent), stigmasterol (23 per cent), β-sitosterol (38 per cent) and another unidentified component, was converted into the benzoates and chromatographed as the preceding fraction. Elution with light petroleum–benzene (9:1) afforded pure β-sitosterol benzoate (0·1 g).

Compounds

Ursolic acid, m.p. 284–288° (from EtOH), $[\alpha]_D$ + 65·3° (*c* 1, EtOH). (Found: C, 78·75; H, 10·34. Calc. for $C_{30}H_{48}O_3$: C, 78·89; H, 10·59%.) Lit.[9] m.p. 289–290°, $[\alpha]_D$ + 64°.

9(11)-*Fernene*, m.p. 168–170°, $[\alpha]_D$ − 16° (*c* 0·4, $CHCl_3$). Lit.[4] m.p. 170–171°, $[\alpha]_D$ − 16·5°.

7-*Fernene*, m.p. 208–210° (from hexane), $[\alpha]_D$ − 26·8° (*c* 1, $CHCl_3$). (Found: C, 87·60; H, 12·47. Calc. for $C_{30}H_{50}$: C, 87·73; H, 12·27%.) Lit.[2c] m.p. 208·5–209·5°, $[\alpha]_D$ − 27°.

22(29)-*Hopene*, m.p. 209–211° (from acetone), $[\alpha]_D$ + 60·3° (*c* 1, $CHCl_3$). (Found: C, 87·55; H, 12·38. Calc. for $C_{30}H_{50}$: C, 87·73; H, 12·27%.) Lit.[2c] m.p. 210–211°, $[\alpha]_D$ + 61°.

Lupeol benzoate, m.p. 258–261° (from hexane), $[\alpha]_D$ + 62·5° (*c* 0·8, $CHCl_3$). Found: C, 83·97; H, 10·40. Calc. for $C_{37}H_{54}O_2$: C, 83·72; H, 10·25%.) Lit.[10] m.p. 265°, $[\alpha]_D$ + 60·1°.

24-*Methylenecycloartanol benzoate*, m.p. 154–156° (from acetone–MeOH), $[\alpha]_D$ + 63·5° (*c* 0·7, $CHCl_3$). (Found: C, 83·54; H, 10·30. Calc. for $C_{38}H_{56}O_2$: C, 83·77; H, 10·36%.) Lit.[11] m.p. 156–157°, $[\alpha]_D$ + 62°.

β-*Sitosterol benzoate*, m.p. 146–147° (from hexane), $[\alpha]_D$ − 13·5° (*c* 1, $CHCl_3$). (Found: C, 83·20; H, 10·24. Calc. for $C_{36}H_{54}O_2$: C, 83·34; H, 10·49%.) Lit.[12] m.p. 146–147°, $[\alpha]_D$ − 13·8°.

β-*Sitosterol*, m.p. 140–142° (from chloroform–MeOH), $[\alpha]_D$ − 36·5° (*c* 1, $CHCl_3$). (Found: C, 83·75; H, 12·30. Calc. for $C_{29}H_{50}O$: C, 83·99; H, 12·15%.) Lit.[12] m.p. 136–137°, $[\alpha]_D$ − 36.6°.

Aliphatic hydrocarbons (GLC, per cent), C_{23} (0·8); C_{24} (0·5); C_{25} (1·7); C_{26} (0·9); C_{27} (6·1); C_{28} (1·6); C_{29} (25·9); C_{30} (2·9); C_{31} (41·5); C_{32} (3·5); C_{33} (14·6).

[8] Although it cannot be ruled out completely that ursolic acid and lupeol might have been derived from the *humus* in which the moss grew, since chestnut leaves contain these two triterpenes along with betulin (results from this laboratory); however, the plant material was well freed from the last traces of *Castanea* leaves, so that it seems highly probable that lupeol and ursolic acid are indeed present in the moss. Moreover, other mosses presently under study also appear to contain ursolic acid.

[9] H. R. ARTHUR and W. H. HUI, *J. Chem. Soc.* 2782 (1954).

[10] I. M. HEILBRON, T. KENNEDY and F. S. SPRING, *J. Chem. Soc.* 329 (1938).

[11] G. OHTA and M. SHIMIZU, *Chem. Pharm. Bull. Japan* **6**, 325 (1958).

[12] E. S. WALLIS and P. N. CHAKRAVORTY, *J. Org. Chem.* **2**, 335 (1937).

Fatty acids (GLC of methyl esters, per cent), myristic (1·0); pentadecanoic (1·0); palmitic (24·5); palmitoleic (2·0); heptadecanoic (1·0); heptadecenoic (0·5); stearic (3·0); oleic (13·0); linoleic (34·0); linolenic + arachidic (16·0); behenic (4·0).

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