Phytochemistry, 1968, Vol. 7, pp. 1705 to 1706. Pergamon Press. Printed in England

SHORT COMMUNICATION

TRITERPENES FROM MOSSES—I.

THE OCCURRENCE OF 22(29)-HOPENE IN *THAMNIUM ALOPECURUM* (L.) BR. EUR. SSP. *EU-ALOPECURUM* GIAC.

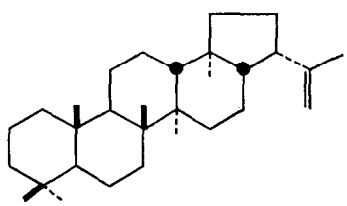
A. MARSILI and I. MORELLI

Istituti di Chimica Farmaceutica e di Chimica Organica dell'Università, 56100 Pisa, Italy

(*Received* 14 *February* 1968)

To OUR knowledge only few triterpenes have so far been isolated from mosses.[1] We wish to report here a preliminary chemical examination of the moss *Thamnium Alopecurum* (L.) Br. eur., ssp. *eu-Alopecurum* Giac., as a part of a wider chemotaxonomic examination of the most common Bryophyta of the Italian flora.

The unsaponifiable portion of the light petroleum extract of the plant was chromatographed over alumina; light petroleum eluted 22(29)-hopene (I). Elution with ethyl ether



(I)

afforded four fractions containing mixtures of alcohols; ergosterol, stigmasterol and β-sitosterol were identified in all fractions by GLC retention times and i.r. spectra. At least two other unidentified alcohols (triterpenes, or more probably sterols) were also present; these are still under study.

The fatty acids obtained from the saponified material were converted into the corresponding methyl esters and analysed by GLC: the presence of acids from C_{12} to C_{28} (some of which unsaturated) was established.

Triterpene hydrocarbons have so far been isolated only from ferns[2] and, in one instance, from a lichen.[3] The occurrence of 22(29)-hopene in *Thamnium* is of interest, as this is the first example of a triterpene hydrocarbon isolated from a moss. A more extensive survey would be desirable, to test the hypothesis that these compounds are characteristic of primitive plants.

[1] R. HEGNAUER, *Chemotaxonomie der Pflanzen*, p. 188. Birkhauser Verlag, Basel (1962).

[2] H. AGETA, K. IWATA and S. NATORI, *Tetrahedron Letters*, 1447 (1963); H. AGETA, K. IWATA and K. YONEZAWA, *Chem. Pharm. Bull. (Tokyo)* **11**, 408 (1963); H. AGETA, K. IWATA and S. NATORI, *Tetrahedron Letters*, 3413 (1964); G. BERTI, F. BOTTARI, B. MACCHIA, A. MARSILI, G. OURISSON and H. PIOTROWSKA, *Bull. Soc. Chim. Fr.* 2359 (1964); G. BERTI, F. BOTTARI, A. MARSILI, I. MORELLI and A. MANDELBAUM, *Chem. Commun.* 50 (1967); G. N. PANDEY and C. R. MITRA, *Tetrahedron Letters*, 4683 (1967).

[3] T. BRUUN, *Acta Chem. Scand.* **8**, 1291 (1954).

EXPERIMENTAL

The dried plant (1 kg), collected in winter, was extracted in a soxhlet apparatus with light petroleum (6 l., b.p. 30–60°) for 40 hr. Concentration of the extract to a small volume led to separation of a wax (0·2 g, λ_{CO} 5·79 μ). Evaporation to dryness gave a brown oil (3·6 g) which was boiled under reflux with 10 per cent NaOH–EtOH (30 ml) for 4 hr. The alkaline soln. was diluted with H_2O and extracted with Et_2O. The extract, on evaporation, afforded 1·2 g unsaponifiable material. From the alkaline soln., by acidification with 2 N H_2SO_4 and new extraction with Et_2O, 1·1 g fatty acids were obtained.

Unsaponfiable Fraction

The unsaponifiable material was dissolved in light petroleum (100 ml, b.p. 30–60°) and chromatographed on neutral alumina (105 g, grade I, column 1·5 × 53 cm). By elution with light petroleum (500 ml) and evaporation of the solvent, a semi-solid residue was obtained. This was crystallized from acetone and from $CHCl_3$–MeOH to afford needles (25 mg), m.p. 211–214°, $[\alpha]_D^{20}$ +59·5°. I.r., $\lambda_{>C=CH_2}$ 6·10, 11·26 μ, NMR, $CH_2{=}C(CH_3)$— δ1·73 (3H, m), 4·75 (2H, m) ppm. The product was identified as 22(29)-hopene (hopene-b) by comparison with an authentic sample.[4]

Benzene did not elute any product; Et_2O was then used as eluent. From the first 300 ml, 0·2 g semi-solid residue (A) was obtained. Then, three fractions of 80 ml each were collected; these contained, respectively, 0·12 (B), 0·18 (C) and 0·09 g (D) residue. Further elution with Et_2O and with Et_2O–MeOH (9:1 v/v) did not yield any other product. The residues A–D contained mixtures of alcohols (i.r.). These were converted into the corresponding trimethylsilyl ethers and gas-chromatographed with a Perkin–Elmer F 20 instrument (column, 3 per cent SE 30 silicone gum rubber,[5] temp. 250°, carrier gas N_2, flow rate 25 ml/min). Approximate compositions (per cent):[6] A, ergosterol (10), stigmasterol (6), β-sitosterol (66), unknown alcohol I (12), unknown alcohol II (5); B, ergosterol (33), stigmasterol (9), β-sitosterol (57); C, ergosterol (39), stigmasterol (19), β-sitosterol (41); D, ergosterol (38), stigmasterol (28), β-sitosterol (33). These mixtures were also identified in part by comparison of their i.r. spectra with those of authentic samples. The i.r. spectrum of fraction A showed bands at 6·10 and 11·25 μ, typical of a $>C{=}CH_2$ group.

Fatty Acids

The fatty acids were esterified with CH_2N_2 in Et_2O, and the mixture of methyl esters was gas-chromatographed with a Perkin–Elmer 800 instrument (column, 20 per cent ethylene glycol succinate on acid-washed Chromosorb W, temp. 210°, carrier gas He, flow rate 40 ml/min). The following acids were identified (per cent): lauric (1·2), myristic (0·6), palmitic (29·0), palmitoleic (2·0), stearic (1·8), oleic (16·0), linoleic (22·0), linolenic (6·0), arachidic (1·2), behenic (1·8), erucic (9·5), lignoceric (3·0), cerotic (2·5), montanic (0·9).

Acknowledgements—The authors wish to express their gratitude to Prof. U. Tosco (Laboratorio Crittogamico dell'Ufficio Sanitario Provinciale, Torino) for the determination of the plant. Thanks are due also to Dr. G. Taponeco (Laboratorio Provinciale d'Igiene, Pisa) and to Dr. P. L. Barili for the gas-chromatographic analyses. This work was supported by a grant from Consiglio Nazionale delle Ricerche.

[4] W. J. DUNSTAN, H. FAZAKERLEY, T. G. HALSALL and E. R. H. JONES, *Croat. Chem. Acta* **29**, 173 (1957).

[5] This column proved to be the most suitable for a good separation of the sterols. Inferior results were obtained by using a neopentyl glycol succinate on Chromosorb W column; anyway, the presence of ergosterol, β-sitosterol and stigmasterol was confirmed in all fractions.

[6] Retention times of known products were compared with those of authentic samples.