Phytochemistry, 1973, Vol. 12, pp. 1759 to 1761. Pergamon Press. Printed in England.

TRITERPENOIDS OF *LYCOPODIUM MEGASTACHYUM*

NOAH MILLER,* CLAUDE HOOTELE† and JEAN-CLAUDE BRAEKMAN†
Service de Chimie Organique, Faculté des Sciences, Université Libre de Bruxelles, Bruxelles, Belgique

(*Received* 15 *October* 1972. *Accepted* 15 *January* 1973)

Key Word Index—*Lycopodium megastachyum*; Lycopodiaceae; triterpenoids; serratane derivatives.

Abstract—Eight triterpenes of the serratane type have been isolated from *Lycopodium megastachyum* Baker. They are serratenediol, 21-*epi*-serratenediol, serratenediol-3-acetate, 21-*epi*-serratenediol-3-acetate, lycoclavanol, tohogenol diacetate, phlegmanol-D and serratenonediol diacetate.

INTRODUCTION

TRITERPENES of the serratane type in which ring-C is seven membered, have been found in several species of *Lycopodium*,[1] in a fern,[2] in the bark of some *Pinus*[3,4] and *Picea*[5–7] species.

We have examined the triterpene constituents of the species *Lycopodium megastachyum* Baker (syn. *Huperzia megastachya* (Baker) Tard) growing in Madagascar‡ and have isolated eight triterpenes, which all belong to the serratenediol family.

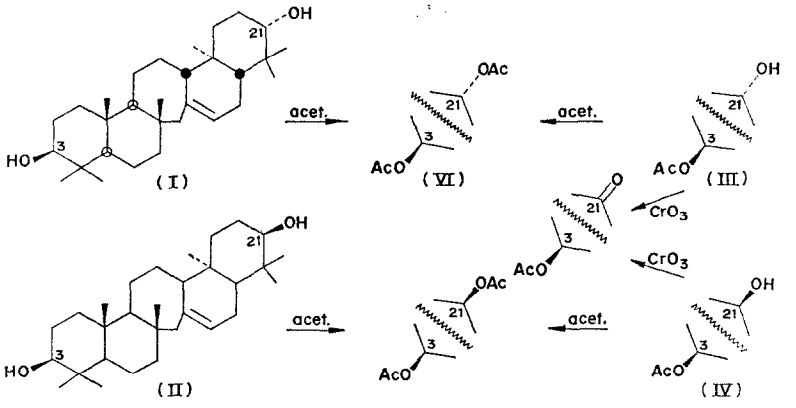
RESULTS

Serratenediol (I), 21-*epi*-serratenediol (II), serratenediol-3-acetate (III), 21-*epi*-serratenediol-3-acetate (IV) and lycoclavanol (V) have been isolated after several chromatographies of the crude light petroleum extract on silicagel. Their identity was settled on the basis of their physical and spectral data. Moreover, derivatives (I) to (IV) were chemically correlated to each other using the reactions depicted in Scheme 1. Compounds (III) and (VI) were shown to be identical with serratenediol diacetate and serratenediol-3-acetate respectively, by direct comparison with authentic samples. Similarly, the acetylated derivative of (V) was proved to be identical with lycoclavanol triacetate (VII).

* Boursier du Gouvernement Belge.
† Chargé de recherches du Fonds National de la recherche scientifique.
‡ The plant material was collected in the Ifody forest near Sabotsy-Anjiro by P. Boiteau.

[1] INUBUSHI, Y., SANO, T. and TSUDA, Y. (1964) *Tetrahedron Letters* 2959; CAMBIE, R. C. and PARNELL, J. C. (1970) *N. Z. J. Sci.* **13**, 108; INUBUSHI, Y., HIBINO, T., HARAYAMA, T. HASEGAWA, T. and SOMANATHAN, R. (1971) *J. Chem. Soc.* **C**, 3109; INUBUSHI, Y., SANO, T. and PRICE J. R. (1967) *Australian J. Chem.* **20**, 387; TSUDA, Y. and HATANAKA, M. (1969) *J. Chem. Soc.* **D**, 1040; BRAEKMAN, J. C., HOOTELE, C. and AYER, W. A. (1971) *Bull. Soc. Chim. Belges* **80**, 83; MILLER, N., HOOTELE, C. BRAEKMAN-DANHEUX, C. and BRAEKMAN, J. C. (1971) *Bull. Soc. Chim. Belges* **80**, 629; BURNELL, R. H., MO, L. and MOINAS, M. (1972) *Phytochemistry* **11**, 2815.
[2] SANO, T. FUJIMOTO, T. and TSUDA, Y. (1970) *J. Chem. Soc.* **D**, 1274.
[3] ROWE, J. W. (1964) *Tetrahedron Letters* 2347.
[4] ROWE, J. W. and BOWER, C. L. (1965) *Tetrahedron Letters* 2745.
[5] ROGERS, I. H. and ROZON, L. R. (1970) *Can. J. Chem.* **48**, 1021.
[6] KUTNEY, J. P. and ROGERS, I. H. (1968) *Tetrahedron Letters* 761.
[7] KUTNEY, J. P., ROGERS, I. H. and ROWE, J. W. (1969) *Tetrahedron* **25**, 3731.

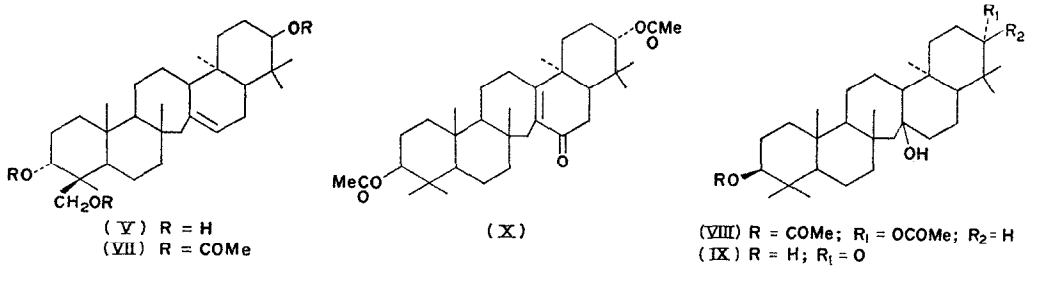
After treatment of the polar fraction of the light petroleum extract with acetic anhydride in pyridine, three more derivatives were separated. Two of them were found to be identical with authentic samples of tohogenol diacetate (VIII) and phlegmanol-D (IX).



SCHEME 1. CHEMICAL CORRELATION BETWEEN (I) TO (IV).

The IR spectrum (ν_{max}^{KBr} at 1730 and 1660 cm^{-1}) and the UV spectrum (λ_{max}^{MeOH} at 255 nm) of the last triterpene isolated ($C_{34}H_{52}O_5$; M = 540; m.p. 313°) strongly suggest the presence of an α,β-unsaturated ketone, leading to the assumption that it could be serratenonediol diacetate.[8] In fact, the compound obtained by oxidation of serratenediol diacetate (VI) with potassium dichromate in glacial acetic acid was proved to be identical with our derivative.

In view of the way derivatives (VIII), (IX) and (X) were obtained it is not known whether their hydroxy groups are free or acetylated in nature.



DISCUSSION

From these results and previous studies on the triterpenoid constituents of the lycopods, it appears that the triterpenes of the serratane type are widely distributed in the genus *Lycopodium*. Moreover it is noteworthy that neither triterpenes of this type, nor alkaloids of the lycopodine type have been found in species of the related genera, *Selaginella* and *Isoetes*. These compounds thus appear to be characteristic of the genus *Lycopodium*, and we think that their presence or absence could be used as a valuable chemotaxonomical criterion to distinguish this genus from the other two.

[8] INUBUSHI, Y., SANO, T. and TSUDA, Y. (1964) *Tetrahedron Letters* 1303.

EXPERIMENTAL

M.ps were determined on a Kofler hot-stage and are uncorrected. The IR spectra were recorded on a Perkin-Elmer 237 double beam instrument, the MS on a RMU-6D Hitachi Perkin Elmer spectrograph and the NMR spectra on a Varian A60 instrument in $CDCl_3$ with TMS as internal standard. TLCs were performed on silica gel G (precoated sheets: Polygram sil G). Detection was accomplished with phosphomolybdic acid.

Acetylation. 5–10 mg of the compound to be acetylated were dissolved in 2 ml pyridine. 1 ml Ac_2O was added to the solution which was then left for 24 hr at room temp. After addition of 10 ml H_2O, the resulting mixture was evaporated to dryness under reduced pressure. The obtained residue was purified by filtration over silica gel (eluent $CHCl_3$) and crystallized.

Isolation and identification. 4·5 kg of dry stems of *L. megastachyum* Baker were powdered and continuously extracted in glass Soxhlets with light petrol. (60–80°). The extract was evaporated to dryness under reduced pressure to give a solid residue (70 g). 0·5 g of this residue were chromatographed on silica gel (50 g; 70–325 mesh) and eluted successively with C_6H_6 (F_1) $CHCl_3$ (F_2), and EtOAc (F_3). Fraction F_1 (400 mg) was placed on a column of silica gel (40 g) and eluted with a gradient of C_6H_6 and $CHCl_3$. The three following compounds were successively eluted.

Serratenediol-3-acetate. M = 484; $C_{32}H_{52}O_3$; m.p. 329–30° (C_6H_6); $[\alpha]_D^{25°}$ −4°(*c* 0·7, $CHCl_3$). The IR spectrum and the R_f ($CHCl_3$–EtOAc, 4:1) were identical to those of an authentic sample. On acetylation a diacetate was obtained (M = 526; $C_{34}H_{54}O_4$; m.p. 336–41° (C_6H_6); $[\alpha]_D^{25°}$ +19° (c 0·7, $CHCl_3$) which was identified as serratenediol diacetate by comparison with an authentic sample (IR, R_f and m.m.p.).

21-epi-*Serratenediol-3-acetate.* M = 484; $C_{32}H_{52}O_3$; m.p. 312–5° (C_6H_6); $[\alpha]_D^{25°}$ −12° (*c* 0·7, $CHCl_3$) IR. ν_{max}^{KBr} at 3440, 1730 and 1240 cm^{-1}. NMR: bs at 0·84 ppm (Me–C), *s* (3H) at 2·01 ppm (Me–COO), *t* (1H) at 3·43 ppm (CH–OH), *bm* (1H) at 4·45 ppm (CH–OAc), *bs* (1H) at 5·33 ppm (HC=C). On acetylation, 21-*epi*-serratenediol diacetate was obtained. M = 526; $C_{34}H_{54}O_4$; m.p. 212–5° (acetone); $[\alpha]_D^{25°}$ −18° (*c* 0·2, $CHCl_3$) IR. ν_{max}^{KBr} at 1735 and 1240 cm^{-1}.

Serratenediol. M = 442; $C_{30}H_{50}O_2$; m.p. 286–8° (C_6H_6). IR. ν_{max}^{KBr} at 3440 cm^{-1}. On acetylation a diacetate is obtained (M = 526; $C_{34}H_{54}O_4$; m.p. 336–8°) which is identified as serratenediol diacetate. (IR, MS, R_f and m.m.p.).

Oxidation of serratenediol-3-acetate and 21-epi-*serratenediol-3-acetate.* To 20 mg serratenediol-3-acetate dissolved in 0·5 ml pyridine 30 mg CrO_3 dissolved in 1·5 ml of pyridine was added. The mixture is stirred for 7 hr at room temp. 10 ml H_2O was added and the resulting solution was extracted with $CHCl_3$ (3 × 50 ml). The organic phase yields serrateneolone acetate which is purified by chromatography on silica gel and by crystallization from the mixture $CHCl_3$–MeOH (M = 482; $C_{32}H_{50}O_3$; m.p. 304–10°. IR. ν_{max}^{KBr} at 1730, 1710 and 1235 cm^{-1}). Similarly, 21-*epi*-serratenediol-3-acetate yielded a ketone (M = 482; m.p. 304–8°) which was found to be identical with the one obtained from serratenediol-3-acetate (IR, MS, R_f and m.m.p.).

Fraction F_2 (70 mg) was placed on a column of silica gel (7 g) and eluted with a gradient of C_6H_6 and $CHCl_3$. The two following compounds were successively eluted.

21-epi-*serratenediol.* M = 442; $C_{30}H_{50}O_2$. On acetylation a diacetate was obtained which is identified as 21-*epi*-serratenediol diacetate (M = 526; $C_{34}H_{54}O_4$; m.p. 215–20°). The IR, MS and the R_f were identical to those of the diacetate obtained from 21-*epi*-serratenediol-3-acetate. No depression of the m.m.p. was observed. *Lycoclavanol.* M = 458; $C_{30}H_{50}O_3$; m.p. 305–6°. On acetylation a triacetate was obtained (M = 584; $C_{36}H_{56}O_6$; m.p. 188–90°) and was identified as lycoclavanol triacetate by comparison with an authentic sample (IR, R_f and m.m.p.).

Fraction F_3 (20 mg) was acetylated using the standard procedure. The solid residue obtained after acetylation was chromatographed on silica gel (3 g) and elution performed with a gradient of C_6H_6 and $CHCl_3$. The three following compounds are successively eluted. *Tohogenol diacetate.* M = 544; $C_{34}H_{56}O_5$; m.p. 305–8°; $[\alpha]_D^{25°}$ +25° (*c* 0·2; $CHCl_3$). The IR spectrum and the R_f were identical with those of an authentic sample of tohogenol diacetate. No depression of the m.m.p. was observed. *Phlegmanol D.* M = 500; $C_{32}H_{52}O_4$; m.p. 292–6° (C_6H_6–light petrol.); $[\alpha]_D^{25°}$ +20° (*c* 0·2, $CHCl_3$). The IR spectrum and the R_f were identical with those of an authentic sample of phlegmanol D. *Serratenonediol diacetate.* M = 540; $C_{34}H_{52}O_5$; m.p. 313°; $[\alpha]_D^{25°}$ +30° (*c* 0·3; $CHCl_3$). IR. ν_{max}^{KBr} at 1730, 1660, 1610 and 1250 cm^{-1}. UV. $\lambda_{max}^{CH_3OH}$ at 255 nm (ϵ = 2900). The IR, MS and the R_f are identical with those of serratenonediol diacetate prepared from serratenediol diacetate using the procedure described by Inubushi.[8] No depression of the m.m.p. was observed.

Acknowledgements—The authors wish to thank Professors R. H. Martin and J. Pecher for their stimulating interest. They also thank the 'Fonds National de la Recherche Scientifique' and the Belgian Government for the award of fellowships. They are indebted to Professor P. Potier who generously furnished the plant material and to Professor Y. Inubushi for the gift of several reference compounds.