# SHORT COMMUNICATION

# TRITERPENOIDS AND OTHER CONSTITUENTS FROM THE FAR-EASTERN SPECIES OF *ALNUS*

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#### (Received 10 March 1971)

Abstract—The following substances were isolated from the unsaponifiable part of the ethereal extracts of the Far-Eastern species of Alnus (A. hirsuta, A. fruticosa, A. mandshurica, A. japonica and A. kamtschatica): heptacosane, a mixture of four aliphatic alcohols, lupenone, glutin-5-cn-3-ol, a mixture of  $\alpha$ - and  $\beta$ -amyrins and  $\beta$ -sitosterol. Moreover, 1,7-diphenylheptan-3,5-diol was isolated for the first time from the extracts of A. fruticosa and A. mandshurica.

### INTRODUCTION

WHILE searching for accessible sources of tetracyclic triterpenes of the dammarane series, we have studied the chemical composition of the leaves of Far-Eastern species of Alnus. Previously, Fischer and Seiler<sup>1</sup> had shown the presence of such triterpenes in Alnus glutinosa. However, their presence in the Far-Eastern species has not previously been demonstrated;  $\beta$ -sitosterol was found to be predominant in the unsaponifiable fraction. A. fruticosa and A. mandshurica also contain considerable amounts of 1,7-diphenylheptan-3,5-diol.

## **RESULTS AND DISCUSSION**

Dry leaves were extracted with ether at room temp., and the resultant extract was then subjected to the usual treatment. The unsaponifiable portion was chromatographed on Al<sub>2</sub>O<sub>3</sub>, and the following substances were isolated: heptacosane, m.p. 59–59.5°, mass-spectrum m/e 280 (M<sup>+</sup>); lupenone, m.p. 164–167°,  $[\alpha]_D^{20} + 64.2°$  (c 0.25; CHCl<sub>3</sub>); glutin-5-en-3-ol, m.p. 211–212°,  $[\alpha]_D^{20} + 54.4°$  (c 0.19; CHCl<sub>3</sub>); and  $\beta$ -sitosterol, m.p. 136–138°,  $[\alpha]_D^{20} - 38°$  (c 1.3; CHCl<sub>3</sub>). None of these substances showed a depressed m.p. on admixture with an authentic sample.

By treating the fraction containing  $\beta$ -sitosterol and aliphatic alcohols with urea, and by subsequent breaking of clathrate compounds with water, the total aliphatic alcohols were obtained and identified by IR and mass spectral data.<sup>2</sup> The mass spectrum showed a pattern characteristic of linear aliphatic compounds. The higher mass values involved a series of peaks with m/e 336, 308, 280 and 252, with relative intensity ratios 1:8:6:3 respectively. We can assume that m/e 336 corresponds to (M<sup>+</sup>-18) of tetracosanol; the peak ratios for the ions (M<sup>+</sup>-18), (M<sup>+</sup>-18-28), (M<sup>+</sup>-18-56) and (M<sup>+</sup>-18-84) of authentic tricosanol\* are 1:0·3:0·1:0·1 respectively. One may therefore conclude that the ions (M<sup>+</sup>-18) of com-

<sup>\*</sup>A sample of tricosanol was kindly supplied by Dr. A. Watanabe.

<sup>&</sup>lt;sup>1</sup> F. G. FISCHER and N. SEILER, Liebigs Ann. Chem. 644, 162 (1961).

<sup>&</sup>lt;sup>2</sup> M. NILSSON, R. RYHAGE and E. VON SYDOV, Acta Chem. scand. 11, 634 (1957).

ponents with molecular weights corresponding to  $C_{22}H_{45}OH$ ,  $C_{20}H_{41}OH$  and  $C_{18}H_{37}OH$  make the principal contribution to the peaks with m/e 308, 280 and 252.

An additional substance with no definite m.p. was isolated from the unsaponifiable fraction. The substance showed one spot in various TLC systems, forming a pinkish colour with a saturated SbCl<sub>3</sub> solution in chloroform and showing absorption at 1650–1665,  $3630 \text{ cm}^{-1}$  in the IR in CCl<sub>4</sub>. However, chromatography on silica gel impregnated with AgNO<sub>3</sub> showed two spots, indicating an isomeric mixture. The presence of peaks with m/e 426 (M<sup>+</sup>), 408 (M<sup>+</sup>-18), 218 (a), 203 (a-C17), 207 (b), 205 (e) and 189 (b-18) in the mass-spectrum of the isolated substance gives grounds for assigning it to the amyrin series.<sup>3</sup>

A sharp increase of the corresponding peaks was observed in GLC when adding corresponding derivatives of  $\alpha$ - and  $\beta$ -amyrin (authentic samples) to the trimethylsilyl derivative of the substance studied.<sup>4</sup> The above-mentioned substances were characteristic of all the species of *Alnus* investigated, differing only quantitatively from one to another.

A substance with m.p. 94-95° (ethyl ether,  $[a]_{D}^{20} - 10^{\circ}$  (C 2·13; CHCl<sub>3</sub>), analysing for  $C_{19}H_{24}O_2$  previously described by Uvarova et al.,<sup>5</sup> was isolated from A. fruticosa and A. mandshurica, IR absorption bands at 3625, 3550, 1610 and 3000-3100 cm<sup>-1</sup> testified to the presence of free and intramolecularly H-bonded hydroxyl groups and aromatic rings. The substance displays no fluorescence in UV, nor does it give a colour with FeCl<sub>3</sub>. Acetylation with acetic anhydride and pyridine yields a chromatographically homogeneous product showing IR absorption at 1745 cm<sup>-1</sup>. A mass spectrum peak with m/e 284 corresponds to  $M^+$  and coincides with the calculated molecular weight. Peaks (m/e 266 and 248) correspond to fragments  $(M^+-18)$  and  $(M^+-36)$  respectively, indicating the presence of two hydroxyl groups in the molecule; fragments with m/e 77 (C<sub>6</sub>H<sub>5</sub><sup>+</sup>) and 91 (C<sub>6</sub>H<sub>5</sub>CH<sub>2</sub><sup>+</sup>) confirm the presence of monosubstituted aromatic rings in the molecule of the substance studied. The latter is not oxidized with periodate. Oxidation of the diol with  $CrO_3$  in pyridine (0-5°) yielded the ketoalcohol, m.p.  $51-52\cdot5^{\circ}$  (light petroleum),  $[a]_{D}^{20} + 1^{\circ}.5$  (C 0.09; CHCl<sub>3</sub>) analysing for  $C_{19}H_{22}O_2$  with IR peaks at 1715 and 3400–3600 cm<sup>-1</sup>. Oxidation of the diol with  $KMnO_4$  in acetone yielded the same ketoalcohol and a mixture of organic acids, subsequently converted into the methylesters. A GLC study of these esters showed the presence of methylphenylpropionate and methyl benzoate. The formation of phenylpropionic acid per se indicates to the presence of a C<sub>6</sub>H<sub>5</sub>--CH<sub>2</sub>--CH<sub>2</sub>--CHOH-- fragment in the original diol. This allows us to conclude that the diol is a 1,7-diphenylheptane-3,5diol, corroborated by the NMR data.

The presence of two monosubstituted aromatic rings (10-H) was confirmed by intense signals at 7.2 ppm. The quintet at 3.91 ppm indicated the presence in the molecule of two symmetrically situated secondary hydroxyls in the  $-CH_2$ -CHOH- $CH_2$ -CHOH- $CH_2$ -CHOH- $CH_2$ -CHOH- $CH_2$ -CHOH- $CH_2$ -fragment. The secondary nature of the hydroxyl groups was corroborated by the NMR spectrum of the acetate in which the quintet shifted to a low field at 4.9 ppm. The protons of the hydroxyl groups gave a singlet at 3 ppm; the singlet vanished in the NMR spectra of the diol and its acetate, recorded in  $CD_3OD$ . The H-atoms of the three methylene groups adjacent to -CHOH- formed a multiplet at 1.50-1.95 ppm, while the protons of the two methylene groups located near the benzene rings were observed as a multiplet at 2.55-2.90 ppm.

<sup>3</sup> H. BUDZIKIEWICZ, J. M. WILSON and C. DJERASSI, J. Am. Chem. Soc. 35, 3687 (1963).

<sup>4</sup> P. CAPELLA, E. FEDELI and M. CIRIMELE, Chem. Ind. 39, 2590 (1963).

<sup>&</sup>lt;sup>5</sup> N. I. UVAROVA, G. I OSHITOK, A. K. DZIZENKO and G. B. ELYAKOV, *Khimija Prirod. Sojedin.* No. 4, 463 (1970).

#### EXPERIMENTAL

The IR spectra were recorded in CHCl<sub>3</sub> and pellets of KBr. The MS were recorded on a 'MX-1303' spectrometer (U.S.S.R.). The NMR spectra were obtained on a 'ZKR-60' spectrometer (Karl Zeiss, Jena) at 60 Mc in CDCl<sub>3</sub>. The methyl esters of the acids were identified on a 'Zvet-3' GLC equipped with a flame-ionizing detector. The 1 m  $\times$  4 mm column was packed with 10% SE-30 Chromosorb W (60–80 mesh), run at 115°.

A similar column with 5% SE-30 on Chromosorb W (60–80 mesh) was used to separate  $\alpha$ - and  $\beta$ -amyrin trimethylsilyl ethers. In both cases, the carrier-gas was argon, and the flow rate—60 ml/min.

Acknowledgements.—We thank Dr. P. G. Gorovoy and Dr. N. S. Pavlova for leaf samples of various species of *Alnus*. Our thanks are also due to Dr. S. E. Odinokov and Dr. S. N. Dzizenko for recording the IR spectra, to Dr. Yu. N. Elkin for the M-S, as well as to Dr. A. K. Dzizenko and Dr. V. V. Isakov for the NMR spectra and their identification.

Key Word Index—Alnus; Betulaceae; triterpenes; lupenone; amyrins;  $\beta$ -sitosterol.