Phytochemistry, 970, Vol. 9, pp. 907 to 909. Pergamon Press. Printed in England.

SHORT COMMUNICATION

BETULINIC ACID FROM *ARBUTUS MENZIESII**

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(*Received* 2 *September* 1969, *in revised form* 2 *October* 1969)

Abstract—The identity of a natural product, previously isolated from *Arbutus menziesii* but incompletely characterized, has been established as betulinic acid.

INTRODUCTION

DURING extraction of the bark of *Arbutus menziesii* (Ericaceae) by the alkaloidal extractive procedure of Djerassi,[1] a large quantity of acetic acid-insoluble solid was obtained. This solid material was a mixture of substances and we decided to elucidate the structure of the most abundant and most polar component of this mixture first. This component had a m.p. of 275–278° and $[\alpha]_D^{23} + 7{\cdot}9°$. Mass spectral data and microanalyses clearly established its molecular formula as $C_{30}H_{48}O_3$. At this juncture, the unpublished work of Lind[2] on the bark of *A. menziesii* came to our attention, and one of the components isolated, but not completely characterized, bore striking resemblance to our compound. Lind's component, designated Am-270, had a m.p. 265–271° and was assigned the molecular formula $C_{50}H_{80}O_5$. The molecular weight of Lind's Am-270 was determined by the Rast method and the formula $C_{50}H_{80}O_5$ is 5/3 of our formula, $C_{30}H_{48}O_3$, determined by mass spectrometry.

RESULTS AND DISCUSSION

The $C_{30}H_{48}O_3$ constituent isolated in the present investigation had a NMR spectrum characteristic of a triterpenoid, as described below.[3]

Triterpenoid (I) contains one double bond as shown by the presence of a 2-proton doublet centered at τ 5·10 and attributed to the terminal methylene protons of an isopropenyl group.[4] This assignment was supported by the observance of bands at 1640 cm^{-1} and 880 cm^{-1} in the i.r. spectrum, attributed to the ν_{C-C} in $CH_2{=}CR_2$ and to the δ_{C-H} (out-of-plane) of the terminal methylene group.[5] The i.r. spectrum of I exhibited additional absorption bands at

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* Taken in part from the M.Sc. Thesis of Henri Martel, University of Victoria, 1968.

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[1] J. A. JOULE, M. OHASHI, B. GILBERT and C. DJERASSI, *Tetrahedron* **21**, 1717 (1965).

[2] J. H. LIND, *Chemical Studies on the Bark of* Arbutus menziesii (*Madrone*), M.S. Thesis, Chemistry Department, University of Oregon, Eugene, Oregon (1957).

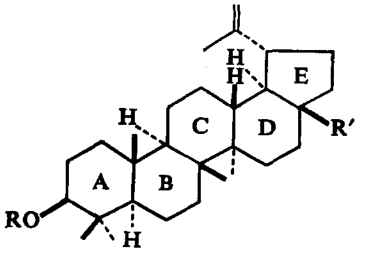
[3] J. C. MANI, *Ann. Chim.* **10**, 533 (1965).

[4] J. M. LEHN and A. VYSTRČYL, *Tetrahedron* **19**, 1733 (1963).

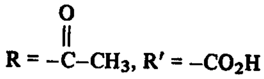
[5] K. NAKANISHI, *Infrared Absorption Spectroscopy—Practical*, p. 24, Holden-Day, Inc., San Francisco (1964).

3480 cm^{-1} and 1043 cm^{-1} due to ν_{O-H} and ν_{C-O} vibrations, and 1685 cm^{-1} due to $\nu_{C=O}$ vibrations, and a carboxylic acid dimer band[6,7] in the 3000–2500 cm^{-1} region. Preparation of the acetate (II) confirmed the presence of an OH group and a carboxylic acid group, since the i.r. spectrum of the acetate (II) no longer showed the ν_{OH} band at 3480 cm^{-1} but still showed $\nu_{C=O}$ at 1688 cm^{-1} and the broad absorption in the 3000–2500 cm^{-1} region. The acetate of I showed an additional intense $\nu_{C=O}$ band at 1733 cm^{-1}. The NMR spectrum of I showed a 15-proton multiplet ranging from τ 8·77–9·16 which accounts for five methyl groups.[3,8] The methyl group of the isopropenyl function resonates as a singlet at τ 8·18 and the proton on the hydroxyl-bearing carbon resonates as a triplet centered at τ 6·53, suggesting that this carbon is adjacent to a single methylene group.[9]

The mass spectrum of compound I was characteristic of a pentacyclic triterpene of the lupane series.[10] The principal peaks in mass range 170–250 were *m/e* 207 ($\Sigma_{40} = 1{\cdot}9\%$), *m/e* 189 ($\Sigma_{40} = 3{\cdot}0\%$), and *m/e* 248 ($\Sigma_{40} = 1{\cdot}4\%$). Other fragments characteristic of the lupane skeleton were found at *m/e* 219 ($\Sigma_{40} = 0{\cdot}75\%$) and 220 ($\Sigma_{40} = 1{\cdot}0\%$).[10]



(I) R = H, R′ = $-CO_2H$

(II) 

$R = -\overset{\displaystyle O}{\overset{\|}{C}}-CH_3,\ R' = -CO_2H$

(III) R = H, R′ = $-CH_2OH$

Upon reduction with $LiAlH_4$, the $C_{30}H_{48}O_3$ constituent (I) was converted to a derivative whose molecular formula was $C_{30}H_{50}O_2$, as shown by high resolution mass spectrometry. This latter conversion indicated that the change $-CO_2H$ to $-CH_2OH$ had occurred, yielding the diol (III). All these chemical and spectroscopic properties can be accounted for if the triterpenoid has structure I, which then should be identical with betulinic acid. Such identity was confirmed by comparison of its i.r., NMR and mass spectra with those of authentic betulinic acid.

EXPERIMENTAL

Melting points were determined on a Bock-Monoscop hot stage microscope and are corrected. I.r. spectra were recorded in KBr pellets or nujol mulls. NMR spectra were obtained in $CDCl_3$ or pyridine-d_5 using TMS as internal standard.

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[6] K. NAKANISHI, *Infrared Absorption Spectroscopy—Practical*, pp. 30–31, Holden-Day, Inc., San Francisco (1964). See Reference 5, pp. 30–31.

[7] L. J. BELLAMY, *The Infrared Spectra of Complex Molecules*, Methuen & Co. Ltd., London (1964).

[8] J. M. LEHN and G. OURISSON, *Bull. Soc. Chim. France*, **10**, 533 (1965).

[9] N. S. BHACCA and D. H. WILLIAMS, *Applications of NMR Spectroscopy in Organic Chemistry, Illustrations from the Steroid Field*, p. 77, Holden-Day, Inc., San Francisco (1964).

[10] H. BUDZIKIEWICZ, J. M. WILSON and CARL DJERASSI, *J. Am. Chem. Soc.* **85**, 3688 (1963).

Triterpenoid (I)

The methanolic extract of 4 kg of the bark of *Arbutus menziesii* was concentrated and extracted with $CHCl_3$ (3 × 1000 ml). The combined $CHCl_3$ extracts were dried over anhydrous Na_2SO_4 and filtered through alumina. Evaporation of the eluate yielded 720 g of dark-brown solid. This solid was suspended in 200 ml methanol, 500 ml HOAc was added, and the resulting mixture was stirred vigorously while water (4 × 500 ml) was added. The slurry was filtered after stirring for 6 hr, yielding 260 g of a greenish solid. The filtrate was stored for further extractions.

A portion (18 g) of the greenish solid was chromatographed on silica gel and eluted successively with petroleum ether, petroleum ether–benzene (4:1), petroleum ether–benzene (1:1), petroleum ether–benzene (1:4), benzene, benzene–$CHCl_3$ (9:1), and $CHCl_3$. Evaporation of the $CHCl_3$ fraction gave 6·23 g (0·15%, based on crude bark) of triterpenoid (I). Crystallization from methanol, followed by vacuum sublimation, afforded triterpenoid (I), m.p. 275–278°, $[\alpha]_D^{23}$ + 7·9° (*c*. 0·057, pyridine). The i.r., NMR and mass spectra were identical with those of an authentic sample of betulinic acid. (Found: C, 78·46; H, 10·49. Calc. for $C_{30}H_{48}O_3$: C, 78·90; H, 10·59.)

Acetate (II)

Acetylation of I with Ac_2O/pyridine afforded the acetate (II), m.p. 269–271°, $[\alpha]_D^{23}$ + 17·3° (*c*. 0·12, $CHCl_3$). (Found: C, 76·23; H, 10·30. Calc. for $C_{32}H_{50}O_4$: C, 77·06; H, 10·10.)

Diol (III)

I was reduced with $LiAlH_4$ in tetrahydrofuran to give the diol (III), m.p. 237–240°. Exact mass measurement: Found: 442·3812. Calc. for $C_{30}H_{50}O_2$: 442·3811.

Acknowledgements—The authors thank the University of Victoria Research Fund and the National Research Council of Canada for generous financial support. Our thanks are also extended to Dr. Leroy H. Klemm, University of Oregon, for providing information with respect to Lind's Am-270 component, and to Dr. T. G. Halsall, the University, Oxford, for supplying an authentic sample of betulinic acid.