

**Fruit pulp.** EtOH concentrate, extrn. with *n*-hexane and Et<sub>2</sub>O, chromatography (silica gel): *Viscous yellow liquid*, IR (ester), sapn. with alc. KOH to  $\alpha$ -amyrin, C<sub>30</sub>H<sub>50</sub>O, m.p., [ $\alpha$ ]<sub>D</sub>, m.m.p.,<sup>6</sup> co-TLC, IR, acetate, m.p. and *fatty acid mixture*  $\rightarrow$  palmitic and stearic acids, co-TLC. *Sitosterol*, C<sub>29</sub>H<sub>50</sub>O, m.p., [ $\alpha$ ]<sub>D</sub>, m.m.p.,<sup>5</sup> IR, co-TLC, acetate, C<sub>31</sub>H<sub>52</sub>O<sub>2</sub>, m.p., IR. *Betulin*, C<sub>30</sub>H<sub>50</sub>O<sub>2</sub>, m.p., [ $\alpha$ ]<sub>D</sub>, IR, m.m.p.,<sup>5</sup> co-TLC, diacetate, C<sub>34</sub>H<sub>54</sub>O<sub>4</sub>, m.p., [ $\alpha$ ]<sub>D</sub>, m.m.p.,<sup>5</sup> IR. *Triterpene acid mixture*, methylation (CH<sub>2</sub>N<sub>2</sub>), chromatography (Al<sub>2</sub>O<sub>3</sub>): Methyl esters of *ursolic* (yield 0.07% of the pulp), *oleanolic* and *betulinic acids*, C<sub>31</sub>H<sub>50</sub>O<sub>3</sub>, identified through m.p., m.m.p.,<sup>5,7,8</sup> [ $\alpha$ ]<sub>D</sub>, IR, MS, their methyl ester acetates, C<sub>33</sub>H<sub>52</sub>O<sub>4</sub>, m.p., m.m.p., IR. Ursolic and oleanolic acids were further confirmed by LiAlH<sub>4</sub> reduction<sup>7</sup> of the former to uvaol, C<sub>30</sub>H<sub>50</sub>O<sub>2</sub>, m.p., IR and SeO<sub>2</sub> oxidation<sup>8</sup> of the latter to  $\Delta^{11,13(18)}$ -diene, UV, respectively.

**Seed.** Stony hard; extrn. EtOH, *n*-hexane soluble fraction, chromatography: *Betulinic acid*, identified as above. Extrn. with *n*-hexane, insignificant traces of fat.

Studies in the various constituents of the fruit pulp and the seed of *Diospyros montana* and *D. peregrina*,<sup>5</sup> reveal that the seed-embryos of both the species hardly yield any fat; betulinic acid is the only constituent present. Sitosterol and betulin are the common constituents of the pulps, in addition *D. montana* pulp yields fatty acid esters of  $\alpha$ -amyrin and ursolic, oleanolic and betulinic acids and the other species yields hexacosane, hexacosanol,  $\beta$ -D-glucoside of sitosterol, gallic acid and a triterpene ketone.

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<sup>6</sup> C. R. MITRA and G. MISRA, *Phytochem.* **4**, 345 (1965).

<sup>7</sup> G. MISRA and C. R. MITRA, *Phytochem.* **7**, 2173 (1968).

<sup>8</sup> S. K. NIGAM and C. R. MITRA, *Planta Med.* **18**, 44 (1970).

**Key Word Index**—*Diospyros montana*; Ebenaceae; steroids; triterpenoids; hydrocarbons.

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## ERICACEAE

### ISOLATION OF ISOPYROSIDE FROM *VACCINIUM VACILLANS*

ALI ASKARI\* and LEONARD R. WORTHEN

College of Pharmacy, University of Rhode Island, Kingston, RI 02881, U.S.A.

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**Plant.** *Vaccinium vacillans* Torr. from Rhode Island U.S.A. **Previous work.** Friedrich first reported<sup>1</sup> the presence of pyroside (6'-acetyl-arbutin) in pear leaves (*Pyrus communis* L.) and mountain cranberry leaves (*Vaccinium vitis-idaea* L.).<sup>2</sup> Haslam *et al.* have confirmed<sup>3</sup>

\* Present address: University of Sulaimani, College of Agriculture, Sulaimani, Iraq.

<sup>1</sup> H. FRIEDRICH, *Pharmazie* **15**, 319 (1960).

<sup>2</sup> H. FRIEDRICH, *Naturwiss.* **48**, 304 (1961).

<sup>3</sup> E. HASLAM, M. O. NAUMANN and G. BRITTON, *J. Chem. Soc.* 5649 (1964).

the structure of pyroside isolated from mountain cranberry leaves. However, Entlicher and Kocourek have reported<sup>4</sup> that the compound present in pear leaves is isopyroside (2'-acetylarbutin) and that the compound present in mountain cranberry is pyroside.

**Isolation.** A sample of fresh frozen leaves of *V. vacillans* was ground and extracted repeatedly with methanol. The extract, after conc. *in vacuo* was chilled and then filtered. The filtrate was extracted with benzene to remove lipids and then chromatographed on polyamide-Celite (2:1), and eluted with H<sub>2</sub>O. The eluate was concentrated *in vacuo* and extracted with EtOAc. This extract was chromatographed on a silica gel HF-254 (TLC) plate in *iso*-BuCOMe-EtOAc-HOAc (3:5:1) satd. with H<sub>2</sub>O. In addition to a spot corresponding to reference arbutin (*R<sub>f</sub>* 0.42), a second spot was detected with *R<sub>f</sub>* 0.55 (Compound I) when the chromatogram was observed in short-wave UV light. The spots developed a purple color when sprayed with diazotized sulphanilic acid (Pauly's reagent). The EtOAc extract was concentrated *in vacuo* and the residue dissolved in a min vol. of hot H<sub>2</sub>O and kept at room temp. for crystallization. The crystals were found to be primarily Compound I (isopyroside) with traces of arbutin. Pure crystals of I were obtained using preparative TLC with the above solvent system.

**Identification.** M.p. 172–174°;  $[\alpha]_D^{22} -22.0^\circ$  (C = 1, H<sub>2</sub>O);  $\lambda_{\text{max}}^{\text{EtOH}}$ , 288 nm ( $\epsilon = 4066$ ); IR peaks at 3520 (free OH), 3200, 2400 (associated OH), 1735 (acetyl carbonyl group), and 1228 (ester group cm<sup>-1</sup>; NMR signals at  $\tau$  3.2 (4 H, complex, aromatic), *ca.* 6.3–6.6 (complex, sugar moiety), 5.31 (1 H, ill-defined quartet of  $\alpha$ -hydrogen of the acetylated hydroxyl group) and 5.07 (1 H, d, C-1' proton signal).

**Results.** The above properties of I were found to be identical to those of the compound isolated from pear leaves in this laboratory, by the above method. The IR spectrum was found to be identical to that published for isopyroside.<sup>4</sup> Previously<sup>4</sup> the structure 2'-acetylarbutin was given tentatively; in this investigation the structure was confirmed by NMR. The 1'-proton signal ( $\tau$  5.07) due to the coupling with 2'-proton, which usually appears as a doublet with almost equal intensities, was shown in this case to be an extremely unbalanced doublet—almost a singlet with a small satellite. This indicates that the chemical shift of the 2'-proton ( $\tau$  5.31) was brought very close to that of the 1'-proton by the presence of an acetyl group on the 2'-OH. Isopyroside was found only in the leaves of *V. vacillans* although samples of six other *Vaccinium* species were examined. *V. vitis-idaea* was not one of those investigated.

<sup>4</sup> G. ENTLICHER and J. KOCOUREK, *Arch. Biochem. Biophys.* **118**, 305 (1967).

**Key Word Index**—*Vaccinium vacillans*; Ericaceae; isopyroside; 2'-acetylarbutin.