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ROOT TRITERPENES OF *VACCINIUM* SPECIES*

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Abstract—The underground portions of *Vaccinium membranaceum* (Dougl.) and *V. parvifolium* (Sm.) were examined for their triterpene content. The first-mentioned ericaceous plant yielded taraxerol, taraxerone, β-sitosterol, and friedel-3-ene. All but the last compound were detected in the other species. This is the first report of friedel-3-ene in the Ericaceae.

INTRODUCTION

THE GENUS *Vaccinium* (Ericaceae), whose chemistry was reviewed recently by Hegnauer,[1] is noted as being rich in triterpene materials. Besides ursolic and oleanolic acids, β-amyrin,[2] friedelin and epifriedelinol[3] occur in several species of this genus, especially in the thick waxy cuticle of their leaves. However, there is a virtual absence of information of the triterpenes in the underground parts of *Vaccinium* species. Indeed, there is only one report on the investigation of triterpene constituents of the rhizomes of an ericaceous plant.[1] This paucity of information, coupled with a desire to continue our work with certain members of the Ericaceae, prompted the investigation of the underground parts of two *Vaccinium* species, *V. membranaceum* (Dougl.) and *V. parvifolium* (Sm.), for their triterpene content.

RESULTS

The powdered roots and rhizomes of *Vaccinium membranaceum* were extracted with light petroleum (95–100°) which yielded a yellow-colored extract. It was found to contain a mixture of sterols/triterpenes when examined by thin-layer chromatography.‡ The mixture was separated by alumina column chromatography (Table 1).

The chloroform fractions (13–18) from the column yielded taraxerol. Its identity was confirmed by preparing its acetate and by oxidation to taraxerone. Taraxeryl acetate prepared from this isolated material could be isomerized to β-amyrin acetate by treating with mineral acid.[4]

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‡ Chromatoplates, unless otherwise specified, were prepared using silica-gel G, according to Stahl, obtained from Brinkmann Instrument, Inc., Westbury, L.I., N.Y., U.S.A.

[1] R. HEGNAUER, *Chemotaxonomie der Pflanzen*, Vol. 4, p. 65. Birkäuser Verlag, Basel (1966).
[2] E. RAMSTAD, *J. Am. Pharm. Assoc., Sci. Ed.* **43**, 236 (1954).
[3] M. YASUE, M. ITAYA, H. OSHIMA and S. FUNAHASHI, *Yakugaku Zasshi* **85**, 553 (1965).
[4] J. M. BEATON, F. S. SPRING, R. STEVENSON and J. L. STEWART, *J. Chem. Soc.* 2131 (1955).

TABLE 1. COLUMN CHROMATOGRAPHY OF PETROLEUM ETHER EXTRACT OF *Vaccinium membranaceum*

Fraction No.	Eluant	Quantity (ml)	R_f of steroidal* component
1	*n*-Hexane	500	—
2–3	*n*-Hexane	1000	0·80
4	*n*-Hexane	500	—
5	Benzene	500	—
6–9	Benzene	2000	0·75
10–12	Benzene	1500	—
13–18	Chloroform	3000	0 60
19–26	Chloroform	4000	0·58, 0 46
27–30	Chloroform	2000	—
31–35	Methanol	2500	—

* Solvent system: Chloroform:acetone (9:1). Detection: 10% w/w $SbCl_3$ in $CHCl_3$ and heat in an oven at 110° for 3–5 min.

The benzene fractions (6–9) from the column were purified by recrystallization from *n*-hexane and then from acetone. The compound was reactive to 2:4 dinitropheynlhydrazine spray reagent on thin-layer plates and showed a strong carbonyl absorption in its i.r.-spectrum. The compound was suspected to be taraxerone and this was verified by the reduction of the isolate to taraxerol. In addition, the i.r.-spectra ($CHCl_3$) of the isolate, as well as taraxerone prepared from authentic taraxerol, were superimposable.

The mixture of two components noted in column fractions 19–26 was easily separated by means of careful chromatography on a second column (Table 2). One of the components was found to be taraxerol while the other was identified as the commonly occurring phytosterol, β-sitosterol. The identity of the latter was established by means of m.p., mixed m.p., and superimposable i.r.-spectra.

TABLE 2 RECHROMATOGRAPHY OF COLUMN FRACTIONS 19–26

Eluant	Fraction No.	Quantity (ml)	Compound
Benzene	1–5	1250	—
Benzene:chloroform (1:1)	6–13	2000	—
Benzene:chloroform (1:2)	14–19	1500	—
Benzene:chloroform (1:3)	20–27	2000	—
Chloroform	28–41	3250	Taraxerol
	42–46	1250	—
Chloroform:ether (3:1)	47–48	500	—
	49–54	1500	β-Sitosterol
	55–57	750	—

The hexane fraction (Table 1, fractions 2–3), upon drying, yielded crystalline material embedded in a waxy mass. The waxy matrix was separated from the crystals which were recrystallized from ethyl acetate. This yielded pure homogeneous crystals melting at 259–261°, $[\alpha]_D + 52{\cdot}2°$ ($CHCl_3$). This compound, which could be revealed as a bright violet spot when its chromatograms were sprayed with antimony trichloride, gave a strongly positive

Liebermann–Burchard color reaction and yellow coloration with tetranitromethane indicating unsaturation. Its i.r.-spectrum revealed the absence of a hydroxyl or carbonyl function and the compound was recovered unchanged following acetylation. The results of elemental analysis excluded any heteroatom in the compound and the molecular ion peak at *m/e* 410 in its mass spectrum required the compound to be $C_{30}H_{50}$. This formula, and an indication of a double bond (inferred from the tetranitromethane test), suggested the compound to be pentacyclic. The mass spectrum also showed a peak at *m/e* 257 suggesting a friedelane derivative with a point of unsaturation.[5]

Isomerization and hydrogenation of the isolate yielded olean-13(18)ene and friedelane, respectively. The mass spectra of both the derivatives were in agreement to those reported by Budzikiewicz *et al.*[6] The isolate and authentic friedel-3-ene gave superimposable i.r.-spectra as did the hydrogenated isolate and an authentic sample of friedelane. Other

TABLE 3. RESULTS OF CO-CHROMATOGRAPHY EXPERIMENTS

Compound	R_f value in solvent system		
	I	II	III
Taraxerol	0·38	0·60	0·55
Isolated taraxerol	0·37	0·62	0·57
Mixture	0·38	0·60	0·55
β-Sitosterol	0·24	0·46	0·48
Isolated β-sitosterol	0·22	0·44	0·47
Mixture	0·23	0·45	0·47
Taraxerone	0·62	0·74	0·80
Isolated taraxerone	0·60	0·76	0·78
Mixture	0·60	0·75	0·80
Friedel-3-ene	0·92	0·02	0·80
Isolated friedel-3-ene	0·90	0·02	0·82
Mixture	0·92	0·03	0·80

I—Skelly-B:ethyl acetate (85:15).
II—benzene:ethyl acetate (3:1).
III—chloroform:acetone (9:1).
Detection: 10% w/w $SbCl_3$ in $CHCl_3$ and heat in an oven at 110° for 3–5 min.

physical constants were in agreement with those reported for friedel-3-ene prepared from friedelin.[7] In addition to this chemical and spectral data, the identity of other materials isolated was confirmed by co-chromatography (Table 3).

The roots and rhizomes of *V. parvifolium* were examined in a like manner. Taraxerol, taraxerone and β-sitosterol were isolated. However, friedel-3-ene was found to be absent in this species.

CONCLUSIONS

Ericaceous plants have been shown to contain a variety of terpenoid products; species of *Vaccinium* being no exception.[1] *V. membranaceum* has now been shown to contain taraxerol,

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[6] H. BUDZIKIEWICZ, J. M. WILSON and CARL DJERASSI, *J. Am. Chem. Soc.* **85**, 3688 (1963).
[7] G. BROWNLIE, F. S. SPRING, R. STEVENSON and W. S. STRACHAN, *J. Chem. Soc.* 2419 (1956).

taraxerone, β-sitosterol, and friedel-3-ene. The last compound was not isolated from *V. parvifolium*.

The existance of friedel-3-ene in nature probably represents a further degeneration of taraxerone via friedelin. Indeed, the occurrence of such compounds was predicted by Brownlie *et al.*[7]

EXPERIMENTAL

Melting points (uncorrected) were determined on a Koffler micromelting point apparatus; optical rotations were measured in $CHCl_3$; i.r.-spectra were recorded using the Beckman Model i.r.-8 recording spectrophotometer; mass spectra were obtained through the courtesy of Professor Egil Ramstad, School of Pharmacy and Pharmacal Sciences, Purdue University, Lafayette, Indiana, U S A.

Collection and Extraction

The roots and rhizomes of *Vaccinum membranaceum* and *V. parvifolium* were collected in Clackamas County, Oregon, in the summer of 1966. The collections were identified by Dr. K. L. Chambers, Curator of the Herbarium, Oregon State University, where voucher specimens were deposited. The plant parts were washed, dried, and ground to a coarse powder and exhaustively extracted with light petroleum. The extracts were concentrated to a syrupy liquid in a rotary evaporator.

Column Chromatography

Alumina columns were developed with *n*-hexane and followed by solvents of increasing polarity. The fractions thus collected were concentrated to a small volume, chromatographed by thin-layer technique using chloroform–acetone (9:1) as a solvent system, and then combined according to the contents of the terpenoidal components The results are summarized in Tables 1 and 2.

Isolation and Identification of Taraxerol

Column fractions 13–18 (Table 1) were combined and concentrated to a small volume and then an equal volume of methanol was added The off-white crystalline material obtained was recrystallized twice from petroleum ether Long needles of taraxerol, m.p. 273–276°, $[\alpha]_D$ 0°, lit. 269–271°, $[\alpha]_D$ 0°,[8] 282–283°, $[\alpha]_D$ 0°,[9] 275–277°,[10] were obtained. Further confirmation was established from the undepressed mixed melting point with an authentic sample and also with co-chromatography (Table 3)

Preparation of Taraxeryl Acetate

Taraxerol (75 mg) was treated as previously reported[11] and yielded white needles, m p. 295–298°, $[\alpha]_D$ + 9°, lit. 296–297°, $[\alpha]_D$ + 8·4°,[8] 304–305°, $[\alpha]_D$ + 9°.[9] The i.r -spectrum ($CHCl_3$) of this compound and an authentic sample of taraxeryl acetate were superimposable.

Isomerization of Taraxeryl Acetate to β-Amyrin Acetate

The isomerization was effected by the method of Beaton *et al.*[4] β-Amyrin acetate, m.p. 236–238°, $[\alpha]_D$ + 82°, lit. 241°, $[\alpha]_D$ + 79°[12] was obtained. The compound did not depress the melting point of β-amyrin acetate (236°).

Oxidation of Taraxerol

Taraxerol (215 mg) was oxidized with CrO_3 in the usual manner. The benzene solution, after drying (Na_2SO_4), was evaporated to dryness and the residue was purified by elution with benzene on a small alumina column. The dried benzene eluate, crystallized from acetone, gave taraxerone, m.p. 237–239°, identical with taraxerone isolated from the plant.

Isolation and Identification of Taraxerone

Column fractions 6–9 (Table 1), upon evaporation, gave a dark-yellow crystalline residue which was crystallized from *n*-hexane and then from acetone. Taraxerone, m p. 239–241°, $[\alpha]_D$ + 8·8°, lit. 240–241°, $[\alpha]_D$ + 11°[9] was obtained. Its identity was verified by co-chromatography (Table 3). The i.r.-spectra of taraxerone obtained by oxidation of taraxerol and that of the isolate were superimposable.

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[9] E KOLLER, A. HIESTAND, P. DIETRICH and O. JEGER, *Helv. Chim. Acta* **33**, 1050 (1950).
[10] C. J. W. BROOKS, *Chem. Ind.* 1178 (1953).
[11] K. SHETH, P. CATALFOMO, L. A. SCIUCHETTI and D. H. FRENCH, *Lloydia* **30**, 78 (1967).
[12] *Merck Index*, 7th Ed., p. 77. Merck and Company, Inc. Rahway, N.J., U.S A. (1960).

Reduction of Taraxerone

$LiAlH_4$ reduction of taraxerone and working-up in the usual manner yielded, after recrystallization from $CHCl_3$–methanol, taraxerol, m.p. 272–274°, $[\alpha]_D$ 0°. Mixed m.p. showed no depression and its i.r.-spectrum was also identical to that of the reference compound.

Isolation and Identification of β-Sitosterol

Dried fractions 19–26 (Table 1) were found to consist of two components. Careful chromatography of this mixture on a smaller alumina column resolved the mixture cleanly. Fractions 49–54 (Table 2) yielded a pale-green crystalline material which was crystallized once from $CHCl_3$–methanol, purified with charcoal and recrystallized twice from ethanol. β-Sitosterol, m.p. 135°, $[\alpha]_D$ −31·3°, was obtained. Superimposable i.r.-spectrum ($CHCl_3$), co-chromatography, and undepressed mixed m.p. with a reference sample of β-sitosterol verified the identity.

Isolation and Identification of Friedel-3-ene

Dried column fractions 2–3 (Table 1) contained prismatic crystals in a waxy matrix. Cold *n*-hexane removed the waxy material leaving the crystals behind and these were crystallized from low boiling light petroleum. Ethyl acetate recrystallization gave solid needles, m.p. 259–261°, $[\alpha]_D$ + 52·2°; friedel-3-ene,[7] m.p. 250–258°, 261–264° (vacuum), $[\alpha]_D$ + 53°. A mixed m.p. with an authentic sample showed no depression. (Found: C, 86·71; H, 12·09. $C_{30}H_{50}$ required: C, 87·54; H, 12·46 per cent.) I.r.-spectrum showed absence of OH and CO absorption. Accordingly, the compound did not react with 2:4 dinitrophenyl-hydrazine and was recovered unchanged after attempting acetylation. The isolate gave a strongly positive Liebermann–Burchard color reaction and yellow color with tetranitromethane. Co-chromatography with an authentic specimen of friedel-3-ene showed no separation of the co-spot. The i.r.-spectra (KBr-pellets) of the isolate as well as the reference compound were identical.

Hydrogenation of Friedel-3-ene

Isolated friedel-3-ene (50 mg) was dissolved in 60 ml cyclohexane. Acetic acid (100 ml) and Pt_2O (80 mg) were added and the mixture was shaken with H_2 (30 lb/in^2) for 18 hr at room temperature. The product, crystallized twice from $CHCl_3$-methanol, yielded friedelane, m.p. 246–248°, $[\alpha]_D$ + 28°, lit. 248–250°, $[\alpha]_D$ + 22°.[7] It did not give any coloration with tetranitromethane. Mixed m.p. with an authentic specimen was undepressed and the i.r.-spectra (KBr-pellets) were identical.

Isomerization of Friedel-3-ene

Friedel-3-ene (50 mg) was isomerized to olean-13(18)-ene by the method of Brownlie *et al.*[7] Repeated crystallizations from $CHCl_3$-methanol mixture yielded olean-13(18)-ene, m.p. 185–186° (lit. 186–187°[13]).

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[13] E. J. Cory and J. J. Ursprung, *J. Am. Chem. Soc.* **77**, 3668 (1955).