839. *Triterpenoids in the Bark of Mountain Ash* (Sorbus aucuparia *L.*).

By WILLIAM LAWRIE, JOHN MCLEAN, and G. R. TAYLOR.

A petroleum extract of the bark of mountain ash (*Sorbus aucuparia* L.) has yielded lupeol and betulin. From an ether extract of the defatted bark, 23-hydroxybetulin has been isolated.

THE bark of the mountain ash or rowan tree (*Sorbus aucuparia* L.) was examined by Danoff and Zellner [1] who reported the isolation, from a petroleum extract, of ceryl alcohol and a triterpenoid compound, $C_{35}H_{60}O$, m. p. 193°, which they designated sorbikortol I (no rotation was quoted). Subsequent extraction with ether, followed by saponification of the extract, produced a second substance sorbikortol II, m. p. 263°, $[\alpha]_D$ −28·9°, which was recognised as an alcohol though crystalline derivatives could not be prepared.

We have examined the extracts of the bark from mountain ash and find that the petroleum extract, when saponified, gives a mixture of alcohols which crystallise from ethyl acetate to give an impure alcohol, m. p. 185—194°, that probably corresponds to sorbikortol I. Acetylation of this mixture followed by chromatography gave lupenyl acetate, identified by mixed m. p. and infrared comparison with an authentic specimen. The constituents of the non-saponifiable fraction were also separated by chromatography on alumina, the early fractions from the column yielding lupenyl acetate on acetylation. Development of the column gave an aliphatic alcohol (probably ceryl alcohol), β-sitosterol, and betulin, the last being converted into its diacetate which was identical with an authentic specimen. We were unable to isolate a pure compound corresponding to sorbikortol I and we suggest that sorbikortol I was in fact a mixture consisting of lupeol and betulin.

The defatted bark was then extracted with ether, and the extract saponified. The neutral non-saponifiable material, which was only sparingly soluble in non-polar solvents,

[1] Danoff and Zellner, *Monatsh.*, 1932, **59**, 307.

was purified by dissolving it in ether containing methanol (1%) and filtering the solution through alumina. Purification of the main fraction from the column afforded a compound, m. p. 259—260°, $[\alpha]_D$ +24·6° (cf. sorbikortol II, m. p. 263°, $[\alpha]_D$ −28·9°, Danoff and Zellner[1]). The compound gave a typical reaction for a pentacyclic triterpene in the Liebermann–Burchard test and showed a strong absorption band at 3278 cm.$^{-1}$ (OH) and bands of medium intensity at 1642 and 880 cm.$^{-1}$ (vinylidene group). The constants of the compound resembled those of betulin[2] (I; R = Me), m. p. 261°, $[\alpha]_D$ +21°, but on admixture with betulin a large depression in melting point was observed. Acetylation of the compound failed to give a crystalline derivative but the resinous product showed strong acetate absorption in the infrared region and no hydroxyl absorption. Hydrolysis of the resinous acetate regenerated the original alcohol. Attempts to form crystalline derivatives of the alcohol with benzoyl chloride and with 3,5-dinitrobenzoyl chloride were equally unsuccessful.

Analysis of the alcohol suggested the molecular formula, $C_{30}H_{50}O_3$. The presence of a double bond was confirmed by low-intensity absorption at 2040 Å and also by a yellow coloration with tetranitromethane.

Hydrogenation of the alcohol gave a fully saturated dihydro-derivative, $C_{30}H_{52}O_3$. This, like the parent, failed to form a crystalline acetyl derivative, but it yielded a crystalline isopropylidene derivative showing infrared bands at 1171, 1112, 1068, and 864 cm.$^{-1}$ (isopropylidenedioxy[3]) and a band at 3390 cm.$^{-1}$ (OH). The dihydro-compound, and its parent, therefore contain three hydroxyl groups, two of them in close proximity.

Treatment of the naturally occurring unsaturated triol, which we shall show to be (I; R = CH_2·OH), with ethanolic hydrogen chloride produced an isomeric but saturated diol (II; R = CH_2·OH), transparent to ultraviolet light and giving no colour with tetranitromethane. This new diol (II; R = CH_2·OH) formed a crystalline diacetate which did not show a hydroxyl band in the infrared spectrum; consequently, protonation of the triol has resulted in a rearrangement involving the double bond and one hydroxyl group. This suggested a rearrangement of the betulin–allobetulin type,[4] which was confirmed by the close correspondence of the infrared spectrum of the diol (II; R = CH_2·OH) with that of allobetulin (II; R = Me). The similarity between the triol (I; R = CH_2·OH) and betulin (I; R = CH_3) was further demonstrated by treatment of the triol with formic acid, a saturated diformate being obtained which gave the diol (II; R = CH_2·OH) on hydrolysis. Under similar conditions betulin (I; R = Me) is converted into allobetulin formate and allobetulin (II; R = Me).[4]

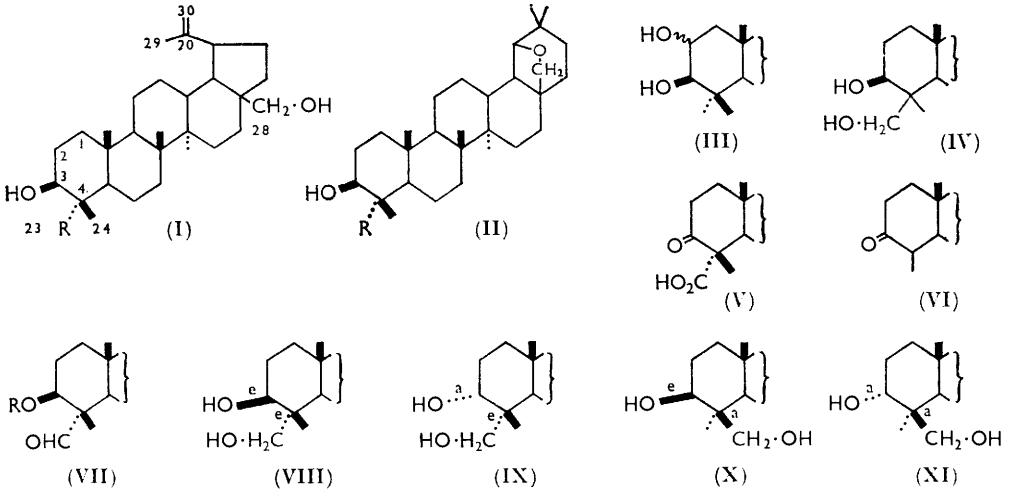
The saturated diol (II; R = CH_2·OH) readily formed crystalline benzylidene and ethylidene derivatives, but the isopropylidene derivative was less stable and decomposed on recrystallisation. The formation of these derivatives indicates that the vicinal hydroxyl groups concerned are not involved in the allomerisation, and if we assume that one of these is at position 3, as in most naturally occurring triterpenes, then the other is likely to be at position 2 (cf. III), 23, or 24 (IV). However, the saturated diol did not react with periodic acid, hence a 1,2-glycol system is not present and structure (III) is excluded.

The diol (II; R = CH_2·OH) with chromium trioxide in pyridine gave two crystalline products which were separated on alumina: a norketone, $C_{29}H_{46}O_2$ (VI), having the carbonyl group in a six-membered ring ($\nu_{max.}$ 1715s cm.$^{-1}$), formed *via* the β-keto-acid (V); and a hydroxy-aldehyde (VII; R = H) ($\nu_{max.}$ 3340, 1738, and 2660 cm.$^{-1}$), whose acetate (VII; R = Ac) was reduced under Huang-Minlon[5] conditions to allobetulin (II; R = Me), identified by comparison with an authentic specimen and as its acetate.

The conversion of the triol (I; R = CH_2·OH) into allobetulin fixes the position of the 3- and 28-hydroxyl groups. No decision regarding the configuration of the 3-hydroxyl

[2] Elsevier's " Encyclopaedia of Organic Chemistry," Vol. XIV, pp. 568, 1133s.
[3] Smith, Smith, and Spring, *Tetrahedron*, 1958, **4**, 127.
[4] Schulze and Pieroh, *Ber.*, 1922, **55**, 2332.
[5] Huang-Minlon, *J. Amer. Chem. Soc.*, 1949, **71**, 3301.

group could be taken at this stage as the strongly alkaline conditions employed in the conversion of the aldehyde (VII; R = Ac) into allobetulin would inevitably lead to an alcohol having the more stable (3β) configuration.[6] Since the third hydroxyl group is at position 23 or 24 (see above), the triol has one of the partial structures (VIII)—(XI). Structure (XI) can be excluded since the 3- and 4-substituents are *trans* and diaxial and would therefore not be expected to form condensation products of this type.[7] To reach a final decision we compared infrared data obtained from the hydroxyallobetulin (II; R = CH_2·OH) with data from diols of known configuration in ring A. Cole and Müller [8] showed, from the infrared absorption of a number of 3-hydroxy-4-hydroxymethyl-triterpenoids in the 2·5—3 μ region, that diols having diaxial groups (type XI) do not show hydrogen-bonding, whereas when the groups are both equatorial (type VIII) or one group is axial and one equatorial (type X), there is intramolecular hydrogen bonding whose strength depends



on the stereochemistry of the groups involved. Our measurements (cm.$^{-1}$) (made with carbon tetrachloride solution and a calcium fluoride prism) are summarised in the Table. They confirm that the 3α,24-diols (diaxial; type XI) do not show a band due to hydrogen bonding; a 3β,24-diol (equatorial : axial; type X) shows a very weak band at 3575 cm.$^{-1}$, whereas a 3β,23-diol (diequatorial; type VIII) shows a band due to hydrogen bonding at 3603 cm.$^{-1}$ which has an intensity similar to that of the free hydroxyl band. Unfortunately a sample of a 3α,23-diol (type IX) was not available but as this is an axial-equatorial diol,

Compound	Free OH	Bonded OH
Urs-12-en-3α,24-diol (type (XI)	3738	—
Olean-12-en-3α,24-diol (type XI)	3738	—
Olean-12-en-3β,24-diol (type X)	3738	3575w
Methyl hederagenin [9] (type VIII)	3738	3603
Hydroxyallobetulin (II, R = CH_2OH)	3738	3603

one would expect its hydrogen-bonding characteristics to resemble those of a diol of type (X) (an equatorial-axial diol). The very striking similarlity in the absorption characteristics in this region of the spectrum of hydroxyallobetulin and methyl hederagenin (type VIII) leads us to conclude that the former is also a 3β,23-diol; the parent triol is therefore 23-hydroxybetulin.

While the melting points of sorbikortol II and 23-hydroxybetulin are in agreement, and the compounds resemble each other in that neither forms a crystalline acetate or benzoate,

[6] Ruzicka and Wirz, *Helv. Chim. Acta*, 1939, **22**, 948; 1940, **23**, 132.
[7] Beton, Halsall, and Jones, *J.*, 1956, 2904.
[8] Cole and Müller, *J.*, 1959, 1224.
[9] Ruzicka, Bischof, Taylor, Meyer, and Jeger, *Coll. Czech. Chem. Comm.*, 1950, **15**, 893.

the rotation quoted by Danoff and Zellner,[1] $[\alpha]_D$ −28·9°, is of the same magnitude as that given by 23-hydroxybetulin, $[\alpha]_D$ +24·6°, but of opposite sign (possibly by a misprint).

Experimental

Rotations were determined for $CHCl_3$ solutions at room temperature unless otherwise stated. Ultraviolet spectra were determined for EtOH solutions. Infrared spectra were determined with a Grubb–Parsons double-beam spectrophotometer, for Nujol mulls unless otherwise stated. Light petroleum refers to the fraction of b. p. 60—80°.

Light-petroleum Extraction of Bark.—Dry crushed bark (10 lb.) of mountain ash was extracted continuously with light petroleum for 15 hr. and the extract (100 g.) was hydrolysed by refluxing with benzene (250 ml.) and potassium hydroxide (60 g.) in methanol (350 ml.). Working up in the usual way through ether gave the non-saponifiable matter (80 g.), a portion of which (10 g.) was chromatographed on alumina (400 g.) from light petroleum–benzene (1 : 1). Development of the column was with benzene, benzene–ether, ether, and ether–methanol, 80 fractions (each 150 ml.) being collected; no appreciable elution occurred until ether was used.

Lupenyl Acetate.—Fractions 25—27, eluted with ether, were acetylated in pyridine–acetic anhydride on the steam-bath for 1 hr. and worked up through ether. Three crystallisations of the product from chloroform–methanol gave lupenyl acetate (1 g.), needles, m. p. 216°, $[\alpha]_D$ +43·2°, identified by mixed m. p. and infrared comparison with an authentic specimen. The mother-liquors yielded a further quantity (0·7 g.) of lupenyl acetate.

Ceryl Alcohol.—Fractions 28—30, eluted with ether, gave an aliphatic alcohol, m. p. 72—73°, probably ceryl alcohol.

β-*Sitosteryl Acetate.*—Fractions 31—33, eluted with ether, were evaporated and the residue crystallised from ethanol. The first crop (0·09 g.) consisted of ceryl alcohol; the mother-liquor deposited impure β-sitosterol (0·8 g.), m. p. 120—130°. Acetylation in pyridine–acetic anhydride and working up as usual gave β-sitosteryl acetate (0·8 g.) as blades (from chloroform–methanol), m. p. and mixed m. p. 127—128°, $[\alpha]_D$ −36°. Infrared comparison with an authentic sample confirmed the identity.

Betulin Diacetate.—Fractions 34—50, eluted with ether containing 0·5% of methanol, gave first crops of material melting in the range 130—138°. These were combined and recrystallised, an additional crop of β-sitosterol (0·9 g.) being obtained. The mother-liquor was taken to dryness in a vacuum, and the residue (1·1 g.) acetylated with pyridine–acetic anhydride. Working up through ether gave crude acetates (1·2 g.) which were chromatographed on alumina (30 g.) from benzene–light petroleum (1 : 2). Elution with benzene–petroleum (2 : 1) gave betulin diacetate (0·4 g.) as needles (from chloroform–methanol), m. p. 223—224°, $[\alpha]_D$ +22°.

Examination of Non-saponifiable Matter by Danoff and Zellner's Procedure.[1]—The non-saponifiable fraction (70 g.) was dissolved in ethyl acetate (200 ml.) and left for 4 days. The crystals (15·1 g.) which separated had m. p. 185—194° (m. p. 194° is recorded [1] for sorbikortol I). Crystallisation of the solid (15·1 g.) from benzene (100 ml.) gave material (7 g.), m. p. 100—130°, which was acetylated in the usual manner and chromatographed on alumina to give β-sitosteryl acetate (1·4 g.), m. p. and mixed m. p. 127—128°, $[\alpha]_D$ −35°. The benzene mother-liquors were evaporated under a vacuum to an oil (5·5 g.) which was chromatographed on alumina (150 g.). Elution with benzene–light petroleum (1 : 3) gave lupenyl acetate (0·4 g.), m. p. 216°, $[\alpha]_D$ +44°, identified as above. Continued elution with the same solvent mixture gave betulin diacetate (0·93 g.), m. p. 223—224°, $[\alpha]_D$ +23°, identified as above.

The non-saponifiable fraction which was soluble in ethyl acetate was recovered by evaporation and a portion (30 g.) in benzene–light petroleum (2 : 1) was chromatographed on alumina (1 kg.). Elution with benzene (2 l.), benzene–ether mixtures (8 l.), and ether (9 l.) produced only intractable material (5·2 g.). Elution with 99 : 1 ether–methanol (1·2 l.) afforded a fraction (12·3 g.) which crystallised from chloroform–methanol to give ceryl alcohol (7·5 g.), m. p. 74—75°. Further elution with ether–methanol (99 : 1; 800 ml.) and crystallisation of the eluted material (3·8 g.) from chloroform–methanol gave β-sitosterol, as plates, m. p. and mixed m. p. 134—135°, $[\alpha]_D$ −34°. The mother-liquors from which β-sitosterol had separated were combined with those from the crystallisation of ceryl alcohol (above), and evaporated. The residue (6·2 g.) was treated with acetic anhydride in pyridine, and a solution of the dry acetylated product in benzene–light petroleum (1 : 4) was chromatographed on alumina (180 g.). Elution with the same solvent gave lupenyl acetate (1·4 g.) as needles (from chloroform–methanol), m. p. and mixed m. p. 215—216°, $[\alpha]_D$ +41°.

Ether-extraction of Bark.—Mountain ash bark (4 lb.) which had been defatted with light petroleum was extracted continously with ether for 24 hr. Removal of the solvent left an extract (80 g.) which was hydrolysed by refluxing 6% methanolic potassium hydroxide (1·5 l.). Working up through ether gave the non-saponifiable material (14 g.).

23-*Hydroxybetulin.*—The non-saponifiable matter (7·3 g.) was chromatographed in 99 : 1 ether–methanol on alumina (200 g.). Elution with ether–methanol (1·2 l.) yielded fractions (2·4 g.) which were bulked and crystallised from ethyl acetate–methanol to give needles, m. p. 246—258°, $[\alpha]_D$ +22·1°, showing strong infrared hydroxyl absorption (3278 $cm.^{-1}$). Acetylation of the alcohol in pyridine–acetic anhydride on the steam-bath (1 hr.) and working up through ether gave a resinous acetate in which the hydroxyl absorption band had been replaced by acetate bands at 1740 and 1248 $cm.^{-1}$. This acetate (0·7 g.) in light petroleum was filtered through a short column of alumina, taken to dryness, and hydrolysed with methanolic potassium hydroxide (100 ml.) for 2·5 hr. Working up through ether and crystallisation from chloroform–light petroleum gave 23-*hydroxybetulin* as needles, m. p. 259—260°, $[\alpha]_D$ +24·6° (Found: C, 78·7; H, 11·1. $C_{30}H_{50}O_3$ requires C, 78·6; H, 11·0%).

20,30-*Dihydro*-23-*hydroxybetulin.*—The resinous acetate (0·54 g.), obtained from 23-hydroxybetulin, was hydrogenated in ether (150 ml.) containing acetic acid (25 ml.) in the presence of platinum oxide (0·4 g.). The resulting dihydro-acetate failed to crystallise. The resinous product was boiled under reflux for 1 hr. with 5% methanolic potassium hydroxide, and the dihydro-alcohol was isolated by means of ether. Filtration of the dried ethereal solution through a short column of alumina and crystallisation from ethanol–light petroleum afforded 20,30-*dihydro*-23-*hydroxybetulin* as needles, m. p. 257—260°, $[\alpha]_D$ −18·4° (from ethanol–chloroform) (Found: C, 78·7; H, 11·5. $C_{30}H_{52}O_3$ requires C, 78·2; H, 11·4%). There was no absorption in the ultraviolet region. Its *isopropylidene derivative*, prepared by means of acetone and concentrated hydrochloric acid (4 drops) and crystallised from aqueous acetone, decomposed at 147° and had $[\alpha]_D$ −19·6° [Found: C, 75·4; H, 10·95. $C_{33}H_{56}O_3,2(CH_3)_2CO$ requires C, 75·9; H, 11·1%].

23-*Hydroxyallobetulin.*—(*a*) 23-Hydroxybetulin (0·33 g.) was refluxed in ethanol (85 ml.) and concentrated hydrochloric acid (15 ml.) for 5 hr. Isolation of the product through ether gave 23-*hydroxyallobetulin* which crystallised from methylene chloride–acetone as plates, m. p. 252—253°, $[\alpha]_D$ +44·3° [Found: C, 76·4; H, 10·7. $C_{30}H_{50}O_3,(CH_3)_2CO$ requires C, 76·7; H, 10·9%].

The diol (0·1 g.) was treated with benzaldehyde (15 ml.) and concentrated sulphuric acid (10 drops) at room temperature overnight. Treatment with sodium carbonate, followed by extraction with ether and evaporation under a vacuum, left an oil which was chromatographed in 1 : 1 light petroleum–benzene on alumina (20 g.). Elution with the same solvent gave the *benzylidene derivative* as needles (from aqueous acetone), m. p. 230° (decomp.), $[\alpha]_D$ +24° (Found: C, 81·3; H, 10·05. $C_{37}H_{54}O_3$ requires C, 81·35; H, 9·95%).

The *ethylidene derivative*, prepared as above by using acetaldehyde, crystallised from aqueous acetone as plates, m. p. 217—222°, $[\alpha]_D$ +48° (Found: C, 79·2; H, 11·1. $C_{32}H_{52}O_3$ requires C, 79·3; H, 10·8%).

23-Hydroxyallobetulin (0·16 g.) in ethanol (25 ml.) was recovered quantitatively after treatment for 24 hr. at room temperature with 15% periodic acid (1 ml.). With acetic anhyride in pyridine at 100° for 1 hr., it gave, after purification on alumina, the *diacetate* as needles (from chloroform–methanol), m. p. 211—212°, $[\alpha]_D$ +68·5°, $\nu_{max.}$ 1740 and 1248 $cm.^{-1}$ (OAc) (Found: C, 75·1; H, 10·0. $C_{34}H_{54}O_5$ requires C, 75·2; H, 10·0%).

(*b*) 23-Hydroxybetulin (0·19 g.) was boiled under reflux (1 hr.) with 98% formic acid (20 ml.). Isolation through ether and crystallisation from aqueous acetone gave 23-*formyloxyallobetulin formate* as needles, m. p. 217—218°, $[\alpha]_D$ +62° (Found: C, 75·1; H, 10·0. $C_{32}H_{50}O_5$ requires C, 74·7; H, 9·8%). Hydrolysis of the diformate (0·3 g.) with 5% methanolic potassium hydroxide (100 ml.) at 100° for 1 hr. gave 23-hydroxyallobetulin as plates (from methylene chloride–acetone), m. p. and mixed m. p. with specimen prepared as in (*a*) above, 252—253°, $[\alpha]_D$ +44°.

Oxidation of 23-*Hydroxyallobetulin.*—A solution of 23-hydroxyallobetulin (0·8 g.) in pyridine (50 ml.) was added to one of chromium trioxide (1·06 g.) in pyridine (60 ml.) and the whole left overnight, then poured into 2N-potassium hydroxide (200 ml.), extracted with ether, and worked up in the usual way to give a gum (0·7 g.) which was chromatographed in benzene on alumina (15 g.). Benzene eluted crystals (0·2 g.) which after two recrystallisations from methylene

chloride–light petroleum afforded 23-*norallobetulone* (VI), m. p. 214—215°, $[\alpha]_D$ +84°, $\nu_{max.}$ 1715 cm.$^{-1}$ (C=O) (Found: C, 81·2; H, 11·2. $C_{29}H_{46}O_2$ requires C, 81·6; H, 10·9%). Subsequent elution of the column with ether and crystallisation of the resinous eluate (0·4 g.) from chloroform–light petroleum gave 23-*oxoallobetulin* (VII) as rosettes, m. p. 243—244°, $[\alpha]_D$ +56·5° (Found: C, 78·8; H, 10·7. $C_{30}H_{48}O_3$ requires C, 78·9; H, 10·6%). 23-Oxoallobetulin (0·17 g.) in pyridine (10 ml.) with acetic anhydride (2 ml.) at 100° for 1 hr. (working up through ether) gave 23-*oxoallobetulin acetate*, plates (from chloroform–methanol), m. p. 252—253°, $[\alpha]_D$ +62·7°, $\nu_{max.}$ 1734, 1245 (OAc) and 2800, 1718 cm.$^{-1}$ (CHO) (Found: C, 76·8; H, 9·8. $C_{32}H_{50}O_4$ requires C, 77·1; H, 10·1%).

Conversion of 23-*Oxoallobetulin Acetate into Allobetulin.*—23-Oxoallobetulin acetate (0·65 g.) in diethylene glycol (80 ml.) and 100% hydrazine hydrate (6 ml.) were heated under reflux for 1 hr. Potassium hydroxide (13 g.) in water (20 ml.) was added to the cooled solution which was again refluxed for 0·5 hr. and then distilled until the vapour-temperature reached 220°. The solution was again boiled under reflux (2·5 hr.), cooled, poured into water, and acidified with 5N-hydrochloric acid. Isolation of the product through ether afforded a dark red oil (0·6 g.) which was chromatographed on alumina (15 g.). Elution with ether and crystallisation of the product from chloroform–light petroleum gave allobetulin as needles, m. p. and mixed m. p. 267—268°, $[\alpha]_D$ +49° {acetate, plates (from chloroform–ethanol), m. p. and mixed m. p. 288—290°, $[\alpha]_D$ +58°}. Infrared comparisons of the synthetic allobetulin and its acetate with authentic specimens of these showed identity.

One of the authors (G. R. T.) thanks the Department of Scientific and Industrial Research for a maintenance grant. Thanks are also extended to Dr. P. Bladon for assistance with the infrared spectra.

THE ROYAL COLLEGE OF SCIENCE AND TECHNOLOGY,
GLASGOW.

[*Received, April 11th,* 1960.]