Triterpenoids. Part XL. The Characterisation of Alnusenone.*

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A compound, $C_{30}H_{48}O$, isolated from black alder bark by Chapon and David (*Bull. Soc. chim. France*, 1953, 333) has again been isolated from the same source. This compound, now named alnusenone, is shown to contain a reactive ketone group and one reactive double bond from which it follows that it is pentacyclic.

The isolation of an alcohol alnulin, and of a ketone protalnulin, from the barks of grey alder (*Alnus incana* L.) and black alder (*A. glutinosa* L.) was described by Zellner *et al.* (Zellner and Röglsperger, *Sitzungsber. Akad. Wiss. Wien*, 1923, **132**, 258; Zellner and Weiss, *ibid.*, 1925, **134**, 312; Fröschl and Zellner, *ibid.*, 1930, **139**, 476). Koller, Hiestand, Dietrich, and Jeger (*Helv. Chim. Acta*, 1950, **33**, 1050) showed that alnulin is identical with taraxerol, an alcohol isolated by Burrows and Simpson (*J.*, 1938, 2042) from the root of dandelion (*Taraxacum officinale*) and by Dunstan, Hughes, and Smithson (*Nature*, 1947, **160**, 577; *Austral. J. Chem.*, 1953, **6**, 321) from the bark of *Litsea dealbata* (*Lauraceae*), and that protalnulin is the related ketone, taraxerone. The constitution of taraxerol has recently been determined (Beaton, Spring, Stevenson, and Stewart, *J.*, 1955, 2131).

Extraction of black alder bark with light petroleum, followed by alkaline hydrolysis of the extract by the method of Koller *et al.* (*loc. cit.*), yielded a non-saponifiable fraction which was partly soluble in ether. A preliminary chromatography of the ether-insoluble fraction gave a mixture of ketones ($[\alpha]_D$ +28°). Crystallisation and chromatography of the mixture gave a homogeneous ketone, $C_{30}H_{48}O$, $[\alpha]_D$ +31°, which we name alnusenone, previously isolated from the same source by Chapon and David (*loc. cit.*). During an examination of two samples of the bark, Chapon and David found that one sample, collected in November, readily yielded taraxerol and taraxerone in agreement with the experience of Koller *et al.* (*loc. cit.*). The corresponding fraction from a second sample of bark, collected in February, consisted mainly of a compound $C_{30}H_{48}O$. A comparison of the constants of this and derived compounds described by these authors with alnusenone and its derivatives shows that the two are identical.

Later fractions obtained by us during the chromatographic separation of the ketone mixture had constants which approximate to those of taraxerone. The presence of taraxerone in the ketone mixture was established by reduction of the latter with lithium aluminium hydride, followed by acetylation, which gave a mixture of acetates from which taraxeryl acetate and alnusenyl acetate (see below) were easily separated.

The homogeneity of alnusenone was demonstrated by the following experiments. Reduction of the ketone with sodium and ethanol yields an alcohol, alnusenol, characterised as acetate, benzoate, and tribromoacetate. Hydrolysis of alnusenyl acetate refurnished alnusenol, oxidation of which by chromic acid at room temperature gave alnusenone; the physical constants of this preparation are identical with those of the ketone isolated from alder bark. Analyses of these derivatives, and particularly that of the tribromoacetate, agree with the molecular formula $C_{30}H_{48}O$ for alnusenone.

Alnusenone contains a reactive carbonyl group, since it readily forms an oxime. It also contains one ethylenic bond because alnusenyl acetate absorbs one mol. of hydrogen when reduced over platinum, yielding alnusanyl acetate. The carbonyl group and the double bond of alnusenone are not conjugated since its ultraviolet spectrum shows absorption only in the ethylenic region together with a weak band in the carbonyl region. The intensity of absorption in the ethylenic region suggests that the double bond is trisubstituted.

In contrast with alnusenyl acetate, alnusanyl acetate does not give a colour with tetranitromethane and does not show ethylenic ultraviolet absorption. Hydrolysis of

* Part XXXIX, preceding paper.

alnusanyl acetate yields alnusanol, oxidised by chromic acid at room temperature to alnusanone. That alnusanone (and, consequently, the parent alnusenone) is a ketone, and not an aldehyde, is established by its stability to chromic acid in acetic acid at room temperature and also by its infrared absorption spectrum (in Nujol) which includes a band at 1702 cm.$^{-1}$ typical of a 6-ring ketone. The infrared absorption spectrum of alnusenone also contains a carbonyl band at 1702 cm.$^{-1}$ (Nujol). Wolff–Kishner reduction of alnusenone gives an unsaturated hydrocarbon, alnusene, $C_{30}H_{50}$, catalytic hydrogenation of which yielded alnusane, also obtained by Wolff–Kishner reduction of alnusanone.

Although we cannot give a direct proof that alnusenone is triterpenoid, its molecular formula and its close association with taraxerone justify its provisional inclusion in this group. If our assessment of the molecular formula of alnusenone is correct, its characterisation as a monoethenoid ketone shows that it is pentacyclic. The physical constants of alnusene (m. p. 181—181·5°, $[\alpha]_D$ +56°, +58°) are similar to those of ψ-taraxastene (heterolupene) (m. p. 182—184°, $[\alpha]_D$ +50°; Jeger, Krüsi, and Ruzicka, *Helv. Chim. Acta*, **1947**, **30**, 1048; Ames, Beton, Bowers, Halsall, and Jones, *J.*, 1954, 1905). The two hydrocarbons differ, however, because the physical constants of taraxastane (Jeger, Krüsi, and Ruzicka, *loc. cit.*) are different from those of alnusane. The constants of alnusane (m. p. 235—236°, $[\alpha]_D$ +27°) are similar to those of lupane-I (m. p. 231—233°, $[\alpha]_D$ +22°; Ames *et al.*, *loc. cit.*); the two hydrocarbons are distinct since a mixture showed a large m. p. depression. We thank Professor E. R. H. Jones, F.R.S., for the sample of lupane-I.

	$[M]_D$						
	Alcohol	Acetate	Benzoate	Ketone	Δ_1	Δ_2	Δ_3
Alnusenol	+260°	+216°	+88°	+132°	−44°	−172°	−128°
Alnusanol	+125°	+ 63°	—	+222°	−62°	—	+ 97°

The constants of alnusane are also similar to those of friedelane. A specimen of the last hydrocarbon was prepared from friedelin by the Wolff–Kishner method and in agreement with Bruun (*Acta Chem. Scand.*, 1954, **8**, 71, 76) friedelane had m. p. 248—250°, $[\alpha]_D$ +22° (*c*, 0·6). Alnusane and friedelane are distinct hydrocarbons since a mixture showed a large depression in m. p.

Alnusenone gives a negative reaction in the Zimmermann test, and in this respect it differs from taraxer-14-en-3-one (taraxerone) and from other 3-oxo-derivatives of the pentacyclic triterpenoids (Barton and de Mayo, *J.*, 1954, 887). The differences between the molecular rotations of alnusenol derivatives (see above) show some striking deviations from those between corresponding derivatives of known 3-hydroxy-triterpenoids.

Experimental

For general instructions see Part XXXVIII (*J.*, 1955, 2606).

Alnusenone from Black Alder Bark.—Finely powdered bark was extracted with light petroleum, and the extracted matter hydrolysed as described by Koller *et al.* (*loc. cit.*). A preliminary chromatography of the ether-insoluble non-saponifiable fraction yielded a solid (eluted by light petroleum–benzene and by benzene) which separated from chloroform–methanol as plates, m. p. 235—237°, $[\alpha]_D$ +28° (*c*, 2·0). This solid (11·5 g.) was crystallised once from light petroleum and twice from benzene (mother-liquors A) to give long blades (1·9 g.), m. p. 241—243°, $[\alpha]_D$ +31° (*c*, 2·0), a solution of which in light petroleum–benzene (20 : 1; 1 l.) was chromatographed on alumina (150 g.). Elution with the same solvent mixture (6 l.) gave fractions of m. p.s 245—246° and $[\alpha]_D$ +31° to +30° (*c*, 1·8—2·0). These fractions were combined and crystallised from benzene to give alnusenone as large thick blades (1·5 g.), m. p. 245—246°, $[\alpha]_D$ +31° (*c*, 2·1), $\lambda_{max.}$ 2950 Å (ε 61), ε 3900 at 2050 Å (Chapon and David, *loc. cit.*, give m. p. 247°, $[\alpha]_D$ +31°) (Found: C, 84·7, 85·1; H, 11·6, 11·4. Calc. for $C_{30}H_{48}O$: C, 84·8; H, 11·4%). The *oxime* separates from ethyl acetate as needles, m. p. 288—290° (decomp.) (Found: C, 82·1; H, 11·0; N, 3·4. $C_{30}H_{49}ON$ requires C, 81·9; H, 11·2; N, 3·2%).

The mother-liquors A were evaporated and the solid chromatographed on alumina. Earlier fractions from the chromatogram furnished pure alnusenone (*ca.* 1·3 g.), m. p. 245—246°, $[\alpha]_D$ +31·5° (*c*, 1·8). The specific rotation of subsequent fractions diminished progressively, the final fractions giving slightly impure taraxerone, m. p. 237—240°, $[\alpha]_D$ +14° (*c*, 2·2).

Alnusenyl Acetate.—(*a*) A solution of alnusenone (150 mg.) in benzene (3 c.c.) and ethano (15 c.c.) was treated, during 5 min., with sodium (1·0 g.), and the mixture refluxed for 1 hr. The product was isolated in the usual way and acetylated by acetic anhydride and pyridine at 90°. The acetylated product, crystallised from chloroform–methanol, furnished alnusenyl acetate as needles, m. p. 235—236°, $[\alpha]_D$ +46° (*c*, 1·0), ε 4500 at 2070 Å (Chapon and David give m. p. 235—236°, $[\alpha]_D$ +41·5°) (Found: C, 81·9; H, 11·2. Calc. for $C_{32}H_{52}O_2$: C, 82·0; H, 11·2%). It gives a yellow colour with tetranitromethane.

(*b*) The ketone mixture described above (1·85 g.; m. p. 235—237°, $[\alpha]_D$ +28°) in dry ether (500 c.c.) was refluxed for 2 hr. with lithium aluminium hydride (2·5 g.). The product was acetylated and then crystallised as in (*a*), giving a first crop (mother-liquor B) of plates (280 mg.), m. p. 302—305°, $[\alpha]_D$ +11·5° (*c*, 0·6), recrystallisation of which from the same solvent gave taraxeryl acetate, m. p. 303—305°, $[\alpha]_D$ +12° (*c*, 1·1) (Found: C, 81·6; H, 11·35. Calc. for $C_{32}H_{52}O_2$: C, 82·0; H, 11·2%). Alkaline hydrolysis of the acetate gave taraxerol, separating as small plates (from chloroform–methanol), m. p. 284—286°, $[\alpha]_D$ ±0° (*c*, 0·5), oxidation of which by chromic acid in acetic acid at room temperature gave taraxerone as prismatic plates (from chloroform–methanol), m. p. 242—244°, $[\alpha]_D$ +9°, +9·5° (*c*, 1·3, 1·4). Koller *et al.* (*loc. cit.*) give m. p. 304—305°, $[\alpha]_D$ +9°, m. p. 282—283°, $[\alpha]_D$ ±0°, and m. p. 240—241°, $[\alpha]_D$ +11°, for taraxeryl acetate, taraxerol, and taraxerone respectively.

Evaporation of the mother-liquor B and crystallisation of the residue from chloroform–methanol gave alnusenyl acetate (910 mg.) as needles, m. p. and mixed m. p. 235—237°, $[\alpha]_D$ +46°, +47° (*c*, 1·0, 1·1).

Alnusenol.—A solution of alnusenyl acetate (600 mg.; m. p. 235—237°) in 3% ethanolic potassium hydroxide was refluxed for 3 hr. Crystallisation of the product from methanol gave alnusenol as fine needles, m. p. 203—205°, $[\alpha]_D$ +61° (*c*, 0·9, 1·0) (Chapon and David give m. p. 195—196°, $[\alpha]_D$ +59·5° for this compound) (Found: C, 84·3; H, 12·1. Calc. for $C_{30}H_{50}O$: C, 84·4; H, 11·8%). Dr. S. David has kindly supplied us with a sample of this alcohol, m. p. 193·5—194·5°, $[\alpha]_D$ +60°. In our apparatus, this has m. p. 198—199°, and a mixture with alnusenol, m. p. 200—202°, $[\alpha]_D$ +61°, had m. p. 199—201°.

Alnusenol (200 mg.), in benzene (10 c.c.) and glacial acetic acid (50 c.c.), was treated at room temperature with a solution of chromium trioxide (34·4 mg.) in glacial acetic acid (6·9 c.c.), added dropwise during 10 min. After 1 hr. at room temperature, the product was isolated in the usual way and crystallised from chloroform–methanol to yield alnusenone as plates, m. p. and mixed m. p. 245—247°, $[\alpha]_D$ +31° (*c*, 1·0) (Found: C, 84·8; H, 11·65%).

Alnusenyl benzoate separates from chloroform–methanol as needles, m. p. 234—235°, $[\alpha]_D$ +16·5° (*c*, 1·0) (Chapon and David, *loc. cit.*, give m. p. 234°, $[\alpha]_D$ +10·7°) (Found: C, 83·9; H, 10·5. Calc. for $C_{37}H_{54}O_2$: C, 83·7; H, 10·5%).

A solution of alnusenol (135 mg.) in benzene (15 c.c.) and pyridine (10 c.c.) at 0° was treated dropwise during 3 min. with a solution of tribromoacetyl bromide (0·5 c.c.) in benzene (5 c.c.). The mixture was kept at 0—5° for 30 min., then at room temperature for 2 days. The filtered solution was treated in the usual manner and the product crystallised from ether–acetone to give *alnusenyl tribromoacetate* as needles, m. p. 223° (decomp.), $[\alpha]_D$ +26° (*c*, 1·2) (Found: C, 54·7; H, 7·2. $C_{32}H_{49}O_2Br_3$ requires C, 54·5; H, 7·0%).

Alnusene.—A mixture of alnusenone (300 mg.), sodium methoxide solution (from 1 g. of sodium and 25 c.c. of methanol), and 100% hydrazine hydrate (3 c.c.) was kept at 205° for 18 hr. (autoclave). The product was purified by chromatography on alumina to give *alnusene* (128 mg.); it separated from chloroform–methanol as elongated plates, m. p. 181—181·5°, $[\alpha]_D$ +56° (*c*, 1·8) (Found: C, 87·4; H, 12·2. $C_{30}H_{50}$ requires C, 87·7; H, 12·3%). It gives a yellow colour with tetranitromethane.

Alnusanyl Acetate.—A solution of alnusenyl acetate (200 mg.) in glacial acetic acid (200 c.c.) was shaken with platinum (from 150 mg. of PtO_2) and hydrogen for 3 hr., hydrogen absorption (*ca.* 1 mol.) then being complete. The product crystallised from chloroform–methanol to yield *alnusanyl acetate* as plates, m. p. 264—265°, $[\alpha]_D$ +11°, +11·5° (*c*, 1·6, 1·7) (Found: C, 81·4; H, 11·5. $C_{32}H_{54}O_2$ requires C, 81·6; H, 11·6%). Alnusanyl acetate was recovered unchanged after treatment of its solution in chloroform at room temperature with a stream of dry hydrogen chloride for 1 hr.

Alnusanol, obtained from the acetate by using 3% methanolic potassium hydroxide, crystallised from chloroform–methanol as fine needles, m. p. 255—256°, $[\alpha]_D$ +28° (*c*, 0·8), unchanged on further recrystallisation (Found: C, 82·3, 82·8; H, 12·25, 12·4. $C_{30}H_{52}O,\frac{1}{2}CH_3{\cdot}OH$ requires C, 82·4; H, 12·25%).

Alnusanone.—Alnusanol (400 mg.), in benzene (100 c.c.) and glacial acetic acid (200 c.c.),

was treated at room temperature with a solution of chromium trioxide (68·4 mg.) in glacial acetic acid (13·7 c.c.), added dropwise during 20 min. After 75 min., the solvent was removed under reduced pressure and the neutral product, isolated in the usual manner, chromatographed in light petroleum (150 c.c.) on alumina (12 g.). Elution with light petroleum (150 c.c.) gave an amorphous solid (44 mg.). Continued washing with light petroleum (400 c.c.) and light petroleum–benzene (1 : 1; 50 c.c.) gave fractions (259 mg.) which crystallised from chloroform–methanol as flattened needles (170 mg.), m. p. 227—230°, $[\alpha]_D$ +51° (*c*, 1·7). Four recrystallisations from the same solvent gave ***alnusanone***, m. p. 228—230°, $[\alpha]_D$ +52° (*c*, 1·7) (Found: C, 84·0; H, 11·8. $C_{30}H_{50}O$ requires C, 84·4; H, 11·8%). Further elution of the column with ether (50 c.c.) gave alnusanol (65 mg.), m. p. and mixed m. p. 254—256°. Alnusanone gives a negative reaction in the Zimmermann test.

Alnusane.—(*a*) Alnusanone (80 mg.), sodium methoxide solution (from 0·5 g. of sodium and 25 c.c. of methanol), and 100% hydrazine hydrate (2 c.c.) were heated in an autoclave at 200° for 22 hr. The product, isolated by means of ether, crystallised from chloroform–methanol as small plates (28 mg.), m. p. 234—236°, $[\alpha]_D$ +30° (*c*, 0·8). Recrystallisation from the same solvent gave *alnusane* as plates, m. p. 235—236°, $[\alpha]_D$ +27° (*c*, 1·6) (Found: C, 87·6; H, 13·1. $C_{30}H_{52}$ requires C, 87·3; H, 12·7%). It does not give a colour with tetranitromethane.

(*b*) A solution of alnusene (65 mg.) in glacial acetic acid (200 c.c.) was shaken with platinum (from 100 mg. of PtO_2) and hydrogen for 1 hr. The product, part of which separated during the reaction, was isolated in the usual manner and crystallised from chloroform–methanol to give alnusane as plates (53 mg.), m. p. and mixed m. p. 234—236°, $[\alpha]_D$ +24° (*c*, 1·5).

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