951. *Infrared Spectra of Natural Products. Part VI.* The Characterization of Equatorial and Axial 3-Hydroxyl Groups in Triterpenoids.*

By (MISS) I. L. ALLSOP, A. R. H. COLE, D. E. WHITE, and R. L. S. WILLIX.

A significant difference in the stretching frequency of equatorial (3629—3630 cm.$^{-1}$) and axial (3637—3639 cm.$^{-1}$) 3-hydroxyl groups in triterpenoids is described, and used to determine the configuration of a hydroxyl group in a new compound. Bands in the 950—1200 cm.$^{-1}$ region are correlated with the stereochemistry of the 3-hydroxy-triterpenoids and their acetates. The intense band near 1240 cm.$^{-1}$ in the acetate spectra is " simple " both for equatorial and axial groups, in contrast to the steroids, where axial acetates give a complex system of bands between 1220 and 1260 cm.$^{-1}$.

THE correlation of infrared absorption frequencies with equatorial or axial orientation of hydroxyl and acetoxyl groups in steroids and simpler cyclic compounds [1-9] has provided a simple method of determining the orientation of these groups in new compounds or in those whose stereochemistry has not previously been fully elucidated.[10,11] The present paper extends these measurements to the tetracyclic and pentacyclic triterpenoids, and draws special attention to the stretching frequencies of the hydroxyl groups.

Hydroxyl Compounds.—In the steroids and decalols, equatorial hydroxyl groups are characterized by a strong band near 1040 cm.$^{-1}$, and axial groups by absorption at somewhat lower frequencies (1000—1034 cm.$^{-1}$). This absorption is due to a stretching vibration of the C–O bond, and the difference in frequency is attributed [2,9] to a difference in the

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* Part V, *J.*, 1956, 1007.

[1] Rosenkrantz, Milhorat, and Farber, *J. Biol. Chem.*, 1952, **195**, 509.
[2] Cole, Jones, and Dobriner, *J. Amer. Chem. Soc.*, 1952, **74**, 5571.
[3] Fürst, Kuhn, Scotoni, and Günthard, *Helv. Chim. Acta*, 1952, **35**, 951.
[4] Rosenkrantz and Skogstrom, *J. Amer. Chem. Soc.*, 1955, **77**, 2237.
[5] Dauben, Holger, and Freeman, *ibid.*, 1952, **74**, 5206.
[6] Hirschmann, *ibid.*, 1952, **74**, 5357.
[7] Rosenkrantz and Zablow, *ibid.*, 1953, **75**, 903.
[8] Jones and Herling, *ibid.*, 1956, **78**, 1152.
[9] Cole, *Fortschr. Chem. org. Naturstoffe*, 1956, **13**, 1.
[10] Cole, *J.*, 1952, 4969.
[11] Aebi, Barton, and Lindsay, *Chem. and Ind.*, 1953, 748.

direction of motion of the carbon atom relative to the *cyclo*hexane ring, with a lower restoring force in the axial compounds. The steroid measurements covered a number of different systems in which the A/B ring junction is *cis* (testane and lumistane series) and *trans* (androstane series), but the compounds available for this study of the triterpenoids all have the *trans* A/B ring junction. Hence the 3α-hydroxyl groups are axial and the 3β-equatorial.

The frequencies and peak intensities of the hydroxyl stretching band and the intense peaks between 950 and 1070 cm.$^{-1}$ for six epimeric pairs of 3-hydroxy-triterpenoids are given in Table 1 and typical examples of the spectra are shown in the Figure.

TABLE 1. *Frequencies* (*cm.*$^{-1}$) *and peak intensities* (*in parentheses*) *of* (i) *the* OH *stretching band* (*in carbon tetrachloride*) *and* (ii) *the intense bands in the range* 950—1070 *cm.*$^{-1}$ (*in carbon disulphide*) *for* 3α- *and* 3β-*hydroxy-triterpeniods.*

	(i)	(ii)
Equatorial		
Urs-12-en-3β-ol (α-amyrin)	3630 (48)	1042 (129), 1026 (129), 993 (133)
Olean-12-en-3β-ol (β-amyrin)	3629 (49)	1042 * 1029 (129), 995 (104)
Lup-20(29)-en-3β-ol (lupeol)	3630 (51)	1040 (131), 1013 (90), 983 (80)
Methyl 3β-hydroxylup-20(29)-en-28-oate (methyl betulate)	3629 (54)	1042 (163), 1031 (138), 983 (138)
9 : 19-*cyclo*Lanost-24-en-3β-ol (*cyclo*artenol)	3630 (56)	1045 (98) { 1020 (114) 1004 (101) } 989 (87)
Lanost-8-en-3β-ol (dihydrolanosterol)	3630 (51)	1025 (233)
Axial		
Urs-12-en-3α-ol (*epi*-α-amyrin)	3638	1068 (106), 1057 (80), 990 (119)
Olean-12-en-3α-ol (*epi*-β-amyrin)	3638 (60)	1069 (118) 992 (93)
Lup-20(29)-en-3α-ol (*epi*lupeol)	3639 (61)	1065 (138) 988 (140)
Methyl 3α-hydroxylup-20(29)-en-28-oate	3637 (60)	1066 (100) 989 (182)
9 : 19-*cyclo*Lanost-24-en-3α-ol	3638 (56)	1065 (97), 1047 (97), 969 (93)
Lanost-8-en-3α-ol	3638 (56)	1063 (91) 975 (91)

* Weak band on side of 1029 cm.$^{-1}$ peak.

Hydroxyl stretching frequencies. Careful measurements of the hydroxyl absorption band indicate that axial groups absorb at a higher frequency (3637—3639 cm.$^{-1}$) than equatorial groups (3629—3630 cm.$^{-1}$). The difference in frequency is small, but is easily detectable when a calcium fluoride or lithium fluoride prism is used in a spectrometer equipped with a thermostat to eliminate changes in prism dispersion, and in single-beam instruments the weak water-vapour spectrum in the background provides a very useful internal calibration. There is virtually no variation of frequency among the six compounds of each type, and this information should prove useful in determining the orientation of 3-hydroxyl groups in new triterpenoids. No similar measurements have been reported on steroids or simpler *cyclo*hexane compounds. The difference is not quite so marked in the menthols,[12] but in these, too, the axial frequency (3632 cm.$^{-1}$) is higher than the equatorial (3627—3628 cm.$^{-1}$).

The hydroxyl apparent molar extinction coefficient [13, 14] [$\varepsilon = (1/cl) \log_{10} (I_0/I)$] has a peak value of approximately 50. This figure can be used for determining the number of hydroxyl groups in an unknown compound, but it should be remembered that maximum extinction coefficients are only additive when the groups absorb at exactly the same frequency and for best results absorption areas should be used. The solutions used in this work were all sufficiently dilute to prevent intermolecular hydrogen bonding.

Bands in the C–O *stretching region.* The spectra in the range 950—1070 cm.$^{-1}$ are somewhat more complex than those of 3-epimeric sterols. The equatorial 3-hydroxy-triterpenoids usually have three intense bands, near 1040, 1025, and 990 cm.$^{-1}$, although lanost-8-en-3β-ol resembles the sterols in having only one intense band in this region (1025 cm.$^{-1}$). *cyclo*Artenol and *cyclo*artanol have an additional band at 1004 and 1012 cm.$^{-1}$, respectively. With inversion to the axial configuration the band near 990 cm.$^{-1}$ remains

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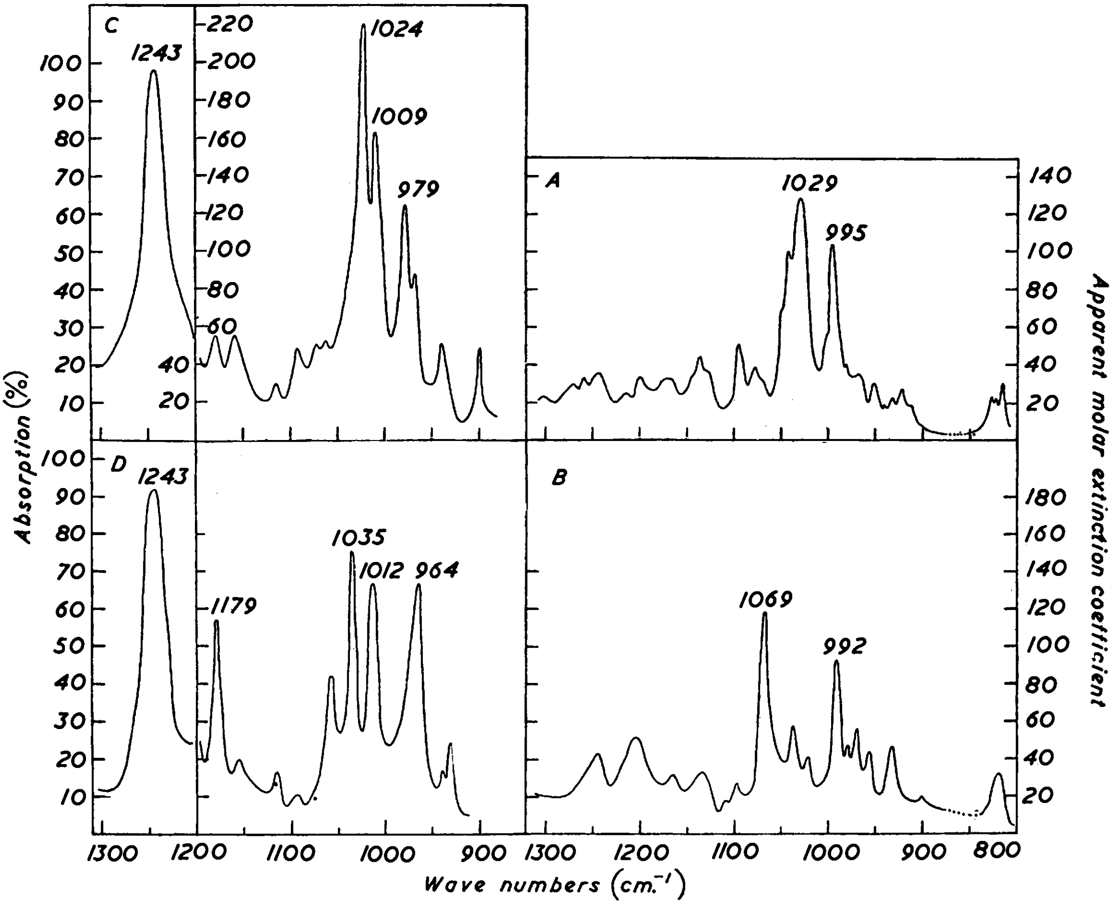
[12] Cole and Jefferies, *J.*, 1956, 4391.

[13] Ramsay, *J. Amer. Chem. Soc.*, 1952, **74**, 72.

[14] Smith and Creitz, *J. Res. Nat. Bur. Stand.*, 1951, **46**, 145.

in the same position, but the other intense absorption moves to 1063—1069 cm.$^{-1}$. Thus it appears that absorption at either 1025 or 1040 cm.$^{-1}$ (equatorial) and 1065 cm.$^{-1}$ (axial) must be identified as that due to the stretching of the C–O bond. This increase in frequency with change from equatorial to axial orientation is opposite to that found for the sterols and must be due to the presence of the *gem*-dimethyl group at $C_{(4)}$. Indeed, the increase in complexity of the spectra in this region compared with those of the sterols is probably due to the increase in mass of this neighbouring centre which may lead to vibrational resonance. It is likely that the fairly intense band near 990 cm.$^{-1}$ is due to a C–C vibration involving this part of the molecule, since Jones, Katzenellenbogen, and Dobriner [15] have shown that in the spectra of the steroidal sapogenins all the intense bands in this region are due to vibrations of bonds near the oxygenated groups.

Infrared spectra of stereoisomeric 3-*hydroxy- and* 3-*acetoxy-triterpenoids* (CS_2 *solutions;* NaCl *prism, double-pass*). *The percent absorption scale applies only to the bands at* 1243 *cm.*$^{-1}$.



(A) *Olean*-12-*en*-3*β*-*ol* (*β*-*amyrin*).
(B) *Olean*-12-*en*-3*α*-*ol* (epi-*β*-*amyrin*).
(C) *Lanost*-8-*en*-3*β*-*yl acetate*.
(D) *Lanost*-8-*en*-3*α*-*yl acetate*.

For determination of the orientation of the 3-hydroxyl group in a triterpenoid the bands at 1065 cm.$^{-1}$ (axial) and 1025—1040 cm.$^{-1}$ (equatorial) are sufficiently specific to leave little doubt. This absorption lies in the range covered by the sodium chloride prism and, where only this prism is available, will be easier to measure than the difference

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[15] Jones, Katzenellenbogen, and Dobriner, *J. Amer. Chem. Soc.*, 1953, **75**, 158.

in hydroxyl stretching absorption described above. Other oxygenated groups (*e.g.*, acetates, see below) often cause fairly strong absorption in this region, but the results for methyl betulate and its $C_{(3)}$-epimer indicate that the two types of hydroxyl orientation can be identified in the presence of a methyl ester group.

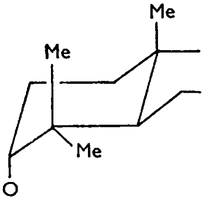
Acetates.—Table 2 gives the frequencies of the intense absorption bands of the epimeric acetates in the range 950—1250 cm.$^{-1}$, and typical spectra are shown in the Figure.

TABLE 2. *Frequencies* (*cm.*$^{-1}$) *and peak intensities* (*in parentheses*) *of the intense bands in the range* 950—1250 *cm.*$^{-1}$ *for* 3α- *and* 3β-*acetoxy-triterpenoids* (*in carbon disulphide*). *The bands near* 1240 *cm.*$^{-1}$ *are all " simple."*

	Equatorial		
Urs-12-en-3β-yl acetate	1240		1023 (223), 1003 (136), 982 (138)
Olean-12-en-3β-yl acetate	1243		1025 (312), 1003 (166), 984 (166)
Lup-20(29)-en-3β-yl acetate	1243		1026 (196), 1015 (157), 978 (200)
Methyl 3β-acetoxylup-20(29)-en-28-oate	1245		1025 (198), 1009 (127), 978 (224)
9 : 19-*cyclo*Lanost-24-en-3β-yl acetate	1243		{1039 (132) 1023 (183)} 977 (124)
Lanost-8-en-3β-yl acetate	1243		1024 (220), 1009 (162), 979 (124)
	Axial		
Urs-12-en-3α-yl acetate	1243	1174 (137)	{1052 (105) 1034 (110)} 1016 (120), 988 (170)
Olean-12-en-3α-yl acetate	1243	1183 (133)	1035 (128), 1016 (121), 986 (123)
Lup-20(29)-en-3α-yl acetate	1243	1180 (133)	1033 (150), 1012 (131), 981 (165)
Methyl 3α-acetoxylup-20(29)-en-28-oate	1245	~1180	1033 (156), 1014 (146), 982 (238)
9 : 19-*cyclo*Lanost-24-en-3α-yl acetate	1243	{1175 (95) 1195 (95)}	1040 (216), 1016 (104), 974 (113)
Lanost-8-en-3α-yl acetate	1243	1179 (115)	1035 (151), 1012 (133), 964 (133)

Absorption near 1240 *cm.*$^{-1}$. The absorption due to one of the C–O stretching vibrations of the acetate group lies near 1240 cm.$^{-1}$. In acetoxy-steroids with the acetoxy-group in ring A [3, 4, 16] this band is usually single and symmetrical for equatorial groups (" simple ") while the absorption due to axial groups consists of a system of two or three peaks between 1220 and 1260 cm.$^{-1}$ (" complex "). An exception to this scheme is cholestan-4α-yl acetate [3] which exhibits complex absorption (1236 and 1247 cm.$^{-1}$).

All the 3-acetoxy-triterpenoids examined exhibit " simple " absorption at 1240—1245 cm.$^{-1}$ regardless of configuration. The lack of complexity of the spectra of the axial group must be attributed to the presence of the $C_{(4)}$-*gem*-dimethyl group since this is the only point of difference between the triterpenoids and steroids in this part of the molecule. Intensity studies at different temperatures led Jones *et al.*[16] to suggest that the " complex " spectra may be due to the presence of rotational isomers. The $C_{(4)}$-methyl groups should not interfere with rotation about the single bonds of the acetate group since they are oriented in different directions (I) and the explanation of the " complex " absorption of the axial acetoxy-steroids may lie in vibrational resonance rather than rotational isomerism. Such resonance could be upset by the increased mass of the $C_{(4)}$ centre in triterpenoids.



(I)

The axial acetates have a fairly intense band (ε ~ 130) near 1180 cm.$^{-1}$ while none of the equatorial acetates has bands with ε >60 at this position. This band is too far away from the peak at 1240 cm.$^{-1}$ to be looked upon as a component of a " complex " system, but should be useful in conjunction with the other spectral regions in determining orientation. With *epi-cyclo*artenyl acetate this absorption is split into two bands of equal intensity (ε = 95) at 1175 and 1195 cm.$^{-1}$. Very strong absorption in this region by the methyl ester group interfered with observation of this band in the spectrum of methyl 3α-acetoxy-lup-20(29)-en-28-oate, although the absorption of the axial compound at 1180 cm.$^{-1}$ is more intense than that of the equatorial.

Absorption near 1000 *cm.*$^{-1}$. Acetates also have fairly intense absorption in the region

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[16] Jones, Humphries, Herling, and Dobriner, *J. Amer. Chem. Soc.*, 1951, **73**, 3215.

960—1040 cm.$^{-1}$ which varies systematically according to the orientation of the group. Equatorial triterpenoid acetates are characterized by a band at 1024—1025 cm.$^{-1}$, to be found at 1033—1035 cm.$^{-1}$ with the axial compound. The shift of this frequency to a higher value for the axial compounds parallels the absorption of the hydroxyl compounds described above, and is again opposite to that described for the steroids.[4, 8] Other strong bands are present at 1003—1016 and 964—988 cm.$^{-1}$ but the frequencies of these are not so clearly related to the stereochemistry.

Table 3 summarizes data useful for determining the orientation of 3-hydroxyl groups in triterpenoids.

TABLE 3. *Summary of band frequencies (cm.$^{-1}$) which are characteristic of equatorial and axial hydroxyl and acetoxyl groups at position* 3 *in triterpenoids.*

	Hydroxyl groups	Acetoxyl groups
Equatorial	3629—3630,* 1013—1031,† 1040—1045 †	1023—1026 †
Axial	3637—3639,* 1063—1068 †	1174—1183,† 1033—1040 †

* Carbon tetrachloride solution. † Carbon disulphide solution.

Application.—The 3-hydroxyl group of dimethyl melaleucate [17] absorbs at 3629 cm.$^{-1}$, while that of its $C_{(3)}$-epimer obtained by oxidation to the ketone and reduction with aluminium *iso*propoxide absorbs at 3636 cm.$^{-1}$. This indicates that the hydroxyl group in the natural product has the β (or equatorial) configuration. The fairly strong absorption of the two methoxycarbonyl groups makes observations in the range 1000—1070 cm.$^{-1}$ rather uncertain, but methyl melaleucate absorbs more strongly near 1040 cm.$^{-1}$ and its $C_{(3)}$-epimer more strongly near 1065 cm.$^{-1}$ which is in accord with Table 1.

EXPERIMENTAL

The infrared spectrometer has been described previously.[18] Solutions in carbon tetrachloride (3-mm. cell; CaF_2 prism) were used for measurement of the hydroxyl stretching frequency and solutions in carbon disulphide (1·3 mm. cell; NaCl prism) for measurements in the region 950—1250 cm.$^{-1}$. Frequencies are accurate to ±1 cm.$^{-1}$ throughout the regions studied, and intensity measurements to ±5%. The effective slit widths at 3630, 1240, and 1000 cm.$^{-1}$ are respectively 6, 4, and 3 cm.$^{-1}$.

Rotations were observed in chloroform solution in 1 dm. tubes. Light petroleum refers to the fraction, b. p. 40—60°. B.D.H. alumina was used for chromatography after being neutralized with nitric acid, washed and standardized.[19] Analyses are by Dr. K. Zimmermann, Melbourne, on samples dried at 100°/0·1 mm. for at least 24 hr. Melting points were determined in evacuated capillaries and are corrected.

Urs-12-*en*-3α-*ol* (epi-α-*Amyrin*).—Urs-12-en-3-one (α-amyrenone) (2·0 g.) was added to a solution of aluminium *iso*propoxide (2·5 g.) in dry propan-2-ol (25 ml.). The mixture was distilled slowly until the distillate was free from acetone (*ca.* 4 hr.), the volume being maintained by adding propan-2-ol when necessary. Finally the solvent was removed *in vacuo* and the residue washed with dilute hydrochloric acid, 5% sodium hydrogen carbonate solution, and water. *Urs*-12-*en*-3α-*ol* (0·54 g.; m. p. 134—137°) was obtained from the crude product in fractions 8—13 (200 ml. each) on elution from alumina (50 g.; activity II) by light petroleum–benzene (9 : 1). Crystallization from aqueous acetone afforded fine needles, m. p. 138°; $[\alpha]_D$ +75° (*c*, 0·98) (Found: C, 84·3; H, 11·9. $C_{30}H_{50}O$ requires C, 84·4; H, 11·8%). Treatment with acetic anhydride and pyridine afforded urs-12-en-3α-yl acetate, m. p. 135°, $[\alpha]_D$ +40° (*c*, 0·72).[20]

From fractions 21—23 (0·44 g.) of the chromatogram α-amyrin, m. p. 186° (from ethanol), was obtained.

Lup-20(29)-*en*-3α-*ol* (epi*Lupeol*).—Lup-20(29)-en-3-one was prepared by oxidation of lupeol with 8N-chromium trioxide in acetone,[21] and on reduction and chromatography as described above afforded *lup*-20(29)-*en*-3α-*ol* in fractions 2—6 eluted by benzene (each 50 ml.). It formed prisms, m. p. 202·5°, $[\alpha]_D$ +15° (*c*, 0·94), from acetone (Found: C, 84·6; H, 12·1. $C_{30}H_{50}O$

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[17] Arthur, Cole, Thieberg, and White, *Chem. and Ind.*, 1956, 926.
[18] Cole and Thornton, *J.*, 1956, 1007.
[19] Brockmann and Schodder, *Ber.*, 1941, **74**, 73.
[20] Büchi, Jeger, and Ruzicka, *Helv. Chim. Acta*, 1946, **29**, 442.
[21] Curtis, Heilbron, Jones, and Woods, *J.*, 1953, 457.

requires C, 84·4; H, 11·8%). The *acetate*, m. p. 163°, $[\alpha]_D$ −1° (*c*, 0·99), formed plates from ether–methanol (Found : C, 81·7; H, 11·3. $C_{32}H_{52}O_2$ requires C, 82·0; H, 11·2%).

From fractions 14—21, lupeol, m. p. 215°, $[\alpha]_D$ +29°, was obtained.

Methyl 3α-*Hydroxylup*-20(29)-*en*-28-*oate*.—Methyl betulonate (prepared with chromium trioxide in acetone[21]) was reduced and chromatographed as above and afforded, in fractions 8—14 eluted from alumina (activity III) with light petroleum–benzene (1 : 1), *methyl* 3α-*hydroxylup*-20(29)-*en*-28-*oate* which formed needles, m. p. 225·5°, $[\alpha]_D$ −13° (*c*, 0·97), from methanol (Found : C, 79·0; H, 10·8. $C_{31}H_{50}O_3$ requires C, 79·15; H, 10·6%). With acetic anhydride and pyridine this afforded *methyl* 3α-*acetoxylup*-20(29)-*en*-28-*oate* which formed needles, m. p. 227°, $[\alpha]_D$ −24° (*c*, 0·92), from aqueous methanol (Found: C, 77·3; H, 10·2. $C_{33}H_{52}O_4$ requires C, 77·3; H, 10·2%).

From fractions 23—29 of the chromatography methyl betulate, m. p. 228°, $[\alpha]_D$ +5°, was obtained.

9 : 19-cyclo*Lanost*-24-*en*-3α-*ol*.—*cyclo*Artenone was reduced by the procedure described above and afforded 9 : 19-cyclo*lanost*-24-*en*-3α-*ol*, in fractions 6—9 eluted with benzene (each 50 ml.) from alumina (activity II). It formed needles, m. p. 84°, $[\alpha]_D$ +39° (*c*, 0·98), from methanol (Found: C, 84·6; H, 11·9. $C_{30}H_{50}O$ requires C, 84·4; H, 11·8%). With acetic anhydride and pyridine it afforded 9 : 19-cyclo*lanost*-24-*en*-3α-*yl acetate*, m. p. 123°, $[\alpha]_D$ +5·5° (*c*, 0·9), which formed prisms from chloroform–methanol (Found : C, 82·2; H, 11·3. $C_{32}H_{52}O_2$ requires C, 82·0; H, 11·2%).

From fractions 13—15, *cyclo*artenol, m. p. 115°, was obtained.

Lanost-8-*en*-3α-*ol*.—Reduction of lanost-8-en-3-one as described above afforded lanost-8-en-3α-ol, in fractions 6—13 eluted with light petroleum–benzene from alumina (activity II). It formed minute prisms, m. p. 139°, $[\alpha]_D$ +49° (*c*, 0·93), from acetone–methanol, and yielded an acetate, m. p. 167·5°, $[\alpha]_D$ −1° (*c*, 0·96) (in good agreement with Marker and Wittle[22]).

The infrared spectrometer was purchased through a generous grant from the Nuffield Foundation, to whom one of us (A. R. H. C.) is indebted for a Fellowship. We thank Professor D. H. R. Barton, Dr. W. Klyne, Dr. H. J. Rodda, Dr. R. G. Curtis, and Dr. R. M. Gascoigne who supplied some of the compounds used and Professor G. L. Adamson for a generous gift of Manila elemi resin.

DEPARTMENT OF CHEMISTRY, UNIVERSITY OF W. AUSTRALIA,
NEDLANDS, W. AUSTRALIA.

[*Received, July* 16*th*, 1956.]

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[22] Marker and Wittle, *J. Amer. Chem. Soc.*, 1937, **59**, 2289.