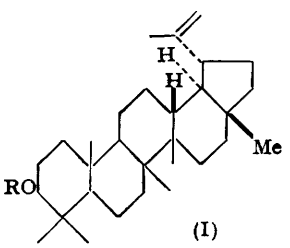
544. *The Chemistry of the Triterpenes. Part XII.* The Action of Formic Acid on Lupeol.*

By T. R. Ames, G. S. Davy, T. G. Halsall, and E. R. H. Jones.

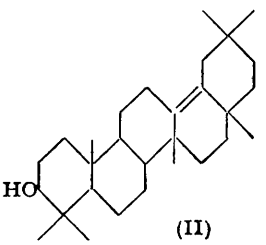
The action of formic acid on lupeol and lupenone has been investigated. When the triterpene is in benzene solution at 20°, the formic acid adds on to the unsaturated system in the same manner as hydrogen chloride (Halsall, Jones, and Meakins, preceding paper) with ring enlargement and the formation of a derivative of 18(α)-oleanane-2"β" : 19(α)-diol. As with the corresponding 19(α)-chloro-compound, this new 19(α)-hydroxy-compound can be converted either into lupenyl acetate by ring contraction or, *via* the corresponding 19-keto-derivative, into 18(α)-oleanane-2"β" : 19(β)-diol 2-acetate and thence to germanicyl acetate. These observations confirm the stereochemical conclusions concerning lupeol drawn in the preceding paper. They are also relevant to the hypothesis put forward by Jeger and Dietrich (*Helv. Chim. Acta*, 1940, **33**, 715) suggesting a common precursor of many of the pentacyclic triterpenes.

A preliminary account of part of this work has already been published (Ames, Davy, Halsall, Jones, and Meakins, *Chem. and Ind.*, 1951, 741).

In continuation of the study of the acidic isomerisation of lupeol (I; R = H) and its derivatives (Ames, Halsall, and Jones, *J.*, 1951, 450), the effect of formic acid has been investigated. Nojd (*Arch. Pharm.*, 1927, **265**, 381) has already studied the action of boiling formic acid on lupeol and has reported the isolation of the formates of two alcohols, α-*allo*lupeol and β-*allo*lupeol, which were suitably characterised. The constants of these compounds and their derivatives do not agree with those of δ-amyrin (II) or lupenol-I and their corresponding derivatives. In the present investigation, by using milder conditions neither α-*allo*lupeol nor β-*allo*lupeol has been isolated, and their nature is not clear.†



(I)



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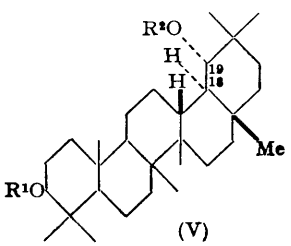

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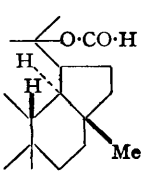

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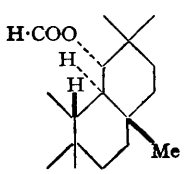

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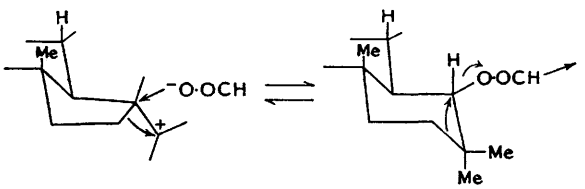


(VI)

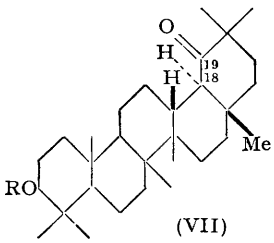
Lupeol reacts with formic acid under very mild conditions. When lupeol or lupenone was shaken in benzene with formic acid for several days, the acid added to the unsaturated centre giving a saturated hydroxy- or keto-formate. Hydrolysis of the formyl group and oxidation of the resulting hydroxyl group led to a ketone. The addition reaction there-

* Part XI, preceding paper.

† *Added in Proof.*—Since this paper was submitted we have obtained full details of the work of Biedebach (*Arch. Pharm.*, 1943, **281**, 49) on the action of boiling formic acid on lupeol. He reports the isolation of α-*allo*lupeol and of a new isomer, γ-*allo*lupeol, but not of β-*allo*lupeol. The constants of γ-*allo*lupeol and of its acetate and benzoate are identical with those of germanicol and its derivatives, and it is extremely likely that γ-*allo*lupeol is germanicol. This is being checked and the nature of α-*allo*lupeol further investigated.

fore gives the formate of a *secondary* alcohol, and consequently partial formula (III) for the formates is excluded. By analogy with the hydrogen chloride addition reaction (Halsall, Meakins, and Jones, preceding paper) partial structure (IV) may be put forward for lupenone formate [19(α)-formyloxy-18(α)-oleanan-2-one *] and lupeol formate [18(α)-oleanane-2"β" : 19(α)-diol 19-formate (V; R^1 = H, R^2 = H·CO)]. This has been confirmed as follows.

Hydrolysis of lupeol formate, or reduction of lupenone formate with lithium aluminium hydride gave a diol [18(α)-oleanane-2"β" : 19(α)-diol (V; R^1 = H, R^2 = H)], acetylation



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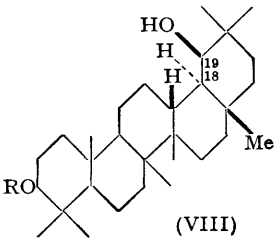

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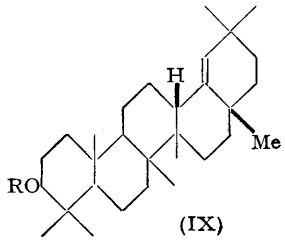

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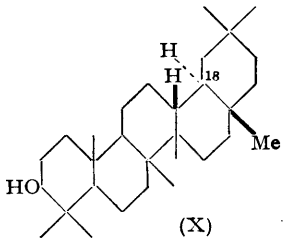

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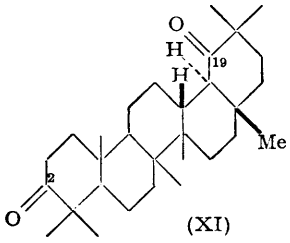

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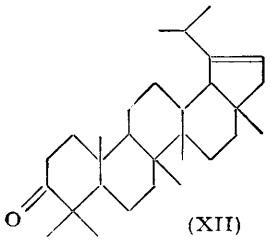

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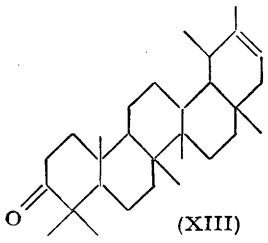

(XIII)

of which led to a monoacetate [18(α)-oleanane-2"β" : 19(α)-diol 2-acetate (V; R^1 = Ac R^2 = H)]. Dehydration of this with phosphorus oxychloride in pyridine gave lupenyl acetate (I; R = Ac), and not germanicyl acetate, the reaction mechanism (cf. VI) presumably being analogous to the dehydrochlorination of lupeol hydrochloride with silver acetate (cf. Halsall, Meakins, and Jones, *loc. cit.*). In this monoacetate, therefore, the 19-hydroxy-group is equatorial and *cis* to the $C_{(18)}$-hydrogen atom. It is known that reduction of the keto-group in derivatives of 19-keto-18(α)-oleanane with lithium aluminium hydride (cf. Davy, Halsall, Jones, and Meakins, *J.*, 1951, 2702; Barton and Holness, *Chem. and Ind.*, 1951, 233) leads to the corresponding polar hydroxy-compound which undergoes *trans*-dehydration to give the 18 : 19-double bond without ring contraction. The monoacetate (V; R^1 = Ac, R^2 = H) was oxidised to the corresponding ketone [19-keto-18(α)-oleanan-2"β"-yl acetate (VII; R = Ac)] which was reduced with lithium aluminium hydride to a diol [18(α)-oleanane-2"β" : 19(β)-diol (VIII; R = H)], different from the original diol. Acetylation of the new diol gave a monoacetate [18(α)-oleanan-2"β" : 19(β)-diol 2-acetate (VIII; R = Ac)], which gave germanicyl acetate (IX; R = Ac), on dehydration with phosphorus oxychloride in pyridine. This dehydration confirms the *trans*-relation of the $C_{(18)}$-hydrogen atom and the $C_{(19)}$-hydroxyl group, which is polar. The series of reactions described above furnish a second method for the conversion of lupeol into germanicol. This is the preferred method, in spite of the greater number of stages as compared with that through lupeol hydrochloride, because of the poor yield of the latter.

* For a discussion of the nomenclature used in this paper see p. 2862.

If the carbonyl group of the ketone (VII; R = Ac) could be reduced either by the Wolff–Kishner or the Clemmensen reaction, 18(α)-oleanan-2"β"-ol (X) (Budziarek, Manson, and Spring, *J.*, 1951, 3336) should result. So far experiments to this end have been unsuccessful. This is not surprising as the similarly situated $C_{(19)}$-keto-group which results on oxidation of siaresinolic acid is not eliminated by Wolff–Kishner reduction (Ruzicka, Grob, and van der Sluys-Veer, *Helv. Chim. Acta*, 1943, **26**, 1218). Reduction of the diketone (XI), obtained by oxidation of the diol (V; $R^1 = R^2 = H$), led to the elimination of the $C_{(2)}$-keto-group, but not of that at $C_{(19)}$, with the formation of 18(α)-oleanan-19-one. In spite of the failure to obtain 18(α)-oleanan-2"β"-ol, which would have provided further evidence for a six-membered ring E in the diol (V; $R^1 = R^2 = H$), evidence on this point has indeed been obtained. The infra-red spectrum of (VII; R = H) in carbon disulphide shows a band at 1700 $cm.^{-1}$ characteristic of a carbonyl group on a six-membered ring.

The epimeric diols (V; $R_1 = R_2 = H$) and (VIII; R = H) are of theoretical interest in connection with Jeger and Dietrich's suggestion (*Helv. Chim. Acta*, 1950, **33**, 715) that α-amyrin, β-amyrin, germanicol (IX; R = H), lupeol (I; R = H), taraxasterol, and ψ-taraxasterol may arise from a common precursor, 2 : 19-dihydroxyoleanane, by dehydration proceeding by different mechanisms. From the results described above it is obvious that stereochemical configuration at $C_{(19)}$ controls the nature of the dehydration products.

The action of formic acid in acetic acid on lupenone at 100° for 16 hours has also been studied. In this case the only product isolated was lupenone-I (Ames, Halsall, and Jones, *loc. cit.*). The structure of this compound is still under investigation. However, since ring enlargement takes place when lupenone is treated with formic acid under very mild conditions such as described above, it is highly probable that ring enlargement also occurs during the formation of lupenone-I. Structure (XII) which has been considered is therefore excluded and lupenone-I is probably best represented by (XIII). Evidence in support of this formulation will be presented later.

Experimental

(M. p.s were determined on a Kofler block and are corrected. Rotations were determined in chloroform. Light petroleum refers to the fraction with b. p. 40—60°. The alumina used for chromatography had an activity of I—II.)

The Action of Formic Acid on Lupenone in Benzene.—Lupenone (5 g.) in dry benzene (30 c.c.) was shaken with formic acid (50 c.c.; 98—100%) for 7 days. The formic acid layer was separated and the benzene solution well washed, dried, and then evaporated. The residue (5·47 g.) was adsorbed from benzene–pentane (1 : 4; 100 c.c.) on to a column of alumina (100 g.) and eluted as follows :

Fraction	Eluant	Vol., c.c.	Wt., g.	M. p.
1—6	Benzene–pentane (1 : 4)	600	2·92	Starting material
7	,, (2 : 5)	140	0·03	—
8	,, (1 : 1)	200	0·52	233—237°
9	Benzene	150	0·96	238—244
10	,,	150	0·35	234—240
11	Ether	200	0·33	228—234

Fractions 1—6 proved to be unchanged lupenone. Fractions 8—10 were crystallised from chloroform–methanol. Recrystallisation of the combined crystalline material (1·34 g.) gave 19(α)-*formyloxy*-18(α)-*oleanan*-2-*one* (IV) as prismatic needles, m. p. 248—251°, $[\alpha]_D^{20}$ +61° (*c*, 1·00), +59° (*c*, 1·63) (Found : C, 78·95; H, 10·75. $C_{31}H_{50}O_3$ requires C, 79·0; H, 10·7%). Crystallisation of fraction 11 from chloroform–methanol gave needles of 19(α)-hydroxy-18(α)-oleanan-2-one (see below), m.p. 228—234°, $[\alpha]_D^{20}$ +24° (*c*, 0·91). The alcohol probably results from the partial hydrolysis of the formate by the alumina.

Hydrolysis of 19(α)-*formyloxy*-18(α)-*oleanan*-2-*one* (IV). The formate (150 mg.) in benzene (10 c.c.) was kept overnight with potassium hydroxide (1·5 g.) and ethanol (15 c.c.) at 20°. The product, after isolation by ethanol extraction, was crystallised from chloroform–methanol and gave 19(β)-*hydroxy*-18(α)-*oleanan*-2-*one* as flat needles, m. p. 229—233°, $[\alpha]_D^{20}$ +21° (*c*, 0·71) (Found : C, 81·5; H, 11·3. $C_{30}H_{50}O_2$ requires C, 81·4; H, 11·4%). A mixture with the material obtained from fraction 11 in the preparation of 19(α)-formyloxy-18(α)-oleanan-2-one showed no depression in m. p.

Reduction of 19(α)-*Formyloxy*-18(α)-*oleanan*-2-*one to* 18(α)-*Oleanane*-2"β" : 19(α)-*diol* (V;

R^1 = H, R^2 = H) *by Lithium Aluminium Hydride.*—The formate (1·04 g.) was dissolved in tetrahydrofuran (40 c.c.), and freshly powdered lithium aluminium hydride (280 mg.) was added; the mixture was then boiled under reflux for 30 minutes. After cooling, the excess of lithium aluminium hydride was decomposed by the careful addition of water, and excess of dilute sulphuric acid was added. The product (1·12 g.), isolated by ethereal extraction, was crystallised from chloroform–methanol giving large needles, m. p. 248—249°. Further recrystallisation gave 18(α)-*oleanane*-2"β"-19(α)-*diol* (608 mg.), m. p. 249—249·5°, $[\alpha]_D^{20}$ −3° (*c*, 1·6), −7° (*c*, 0·73) (Found: C, 81·0; H, 11·65. $C_{30}H_{52}O_2$ requires C, 81·0; H, 11·8%). This diol (377 mg.) was acetylated in pyridine (15 c.c.) with acetic anhydride (30 c.c.) at 100° for 40 minutes, and the warm mixture was then added slowly to an excess of water. The product, isolated by extraction with benzene–ether (1 : 1), was adsorbed from pentane–benzene (1 : 1; 40 c.c.) on to a column of alumina (50 g.). Elution with benzene–ether (1 : 1; 350 c.c.) gave material (374 mg.) which, when crystallised from chloroform–methanol, gave 18(α)-*oleanane*-2"β" : 19(α)-*diol* 2-*acetate* (V; R^1 = Ac, R^2 = H) as needles (165 mg.), m. p. 249—250°, $[\alpha]_D^{20}$ +7° (*c*, 1·2) (Found: C, 78·9; H, 11·5. $C_{32}H_{54}O_3$ requires C, 78·9; H, 11·2%).

Dehydration of 18(α)-*Oleanane*-2"β" : 19(α)-*diol* 2-*Acetate with Phosphorus Oxychloride in Pyridine.*—The diol monoacetate (130 mg.) was dissolved in dry pyridine (15 c.c.), and phosphorus oxychloride (3 c.c.) was added. The solution was refluxed for 2 hours, cooled, and added cautiously to excess of water. The product (142 mg.), isolated by benzene extraction, was adsorbed from pentane–benzene (1 : 1; 20 c.c.) on to a column of alumina (10 g.). Elution with pentane–benzene (1 : 1; 25 c.c.) gave a fraction (127 mg.) which formed flat needles, m. p. 205—207°, from chloroform–methanol. Two recrystallisations from the same mixture gave lupenyl acetate, m. p. 210—214° undepressed on admixture of the sample with an authentic specimen (m. p. 213—215°), $[\alpha]_D^{20}$ +43·5° (*c*, 0·65).

The Addition of Formic Acid to Lupeol.—Lupeol (10 g.) in benzene (120 c.c.) was shaken with formic acid (150 c.c.; 98—100%) at 20° for 7 days. After separation of the formic acid layer the benzene solution was well washed with water and evaporated. The residue was heated under reflux for 1 hour with potassium hydroxide (10 g.) in ethanol (100 c.c.) and benzene (20 c.c.), and the product, isolated by benzene extraction, was acetylated with acetic anhydride in pyridine for 16 hours at 20°. The material (9·1 g.) isolated with benzene was dissolved in benzene–*n*-pentane (7 : 3; 100 c.c.) and adsorbed on a column of alumina (100 g.). The fraction (1·86 g.) eluted with benzene–ether (1 : 1; 300 c.c.) was crystallised from chloroform–methanol giving 18(α)-oleanane-2"β" : 19(α)-diol 2-acetate (V; R^1 = Ac, R^2 = H) as needles (1·19 g.), m. p. 251—256°. A considerable quantity of material, believed to be the diacetate, was obtained from the earlier fractions eluted with benzene.

Oxidation of 18(α)-*Oleanane*-2"β" : 19(α)-*diol.*—The diol (100 mg.) was dissolved in benzene (10 c.c.), and a solution of chromic acid (100 mg.) in acetic acid (8 c.c.), acetone (15 c.c.), and water (1 c.c.) was added with shaking. After 1 hour at 20° dilution with water and extraction with benzene yielded a product which was dissolved in benzene (10 c.c.) and adsorbed on a column of alumina (40 g.). The material (88 mg.), eluted by benzene (350 c.c.), was crystallised from chloroform–methanol giving needles (41 mg.), m. p. 245—249·5°. Recrystallisation of these from the same solvent gave 18(α)-*oleanane*-2 : 19-*dione* (XI) as shining needles, m. p. 249—252°, $[\alpha]_D^{20}$ +70° (*c*, 0·64) (Found: C, 82·0; H, 11·2. $C_{30}H_{48}O_2$ requires C, 81·75; H, 10·95%). Light absorption in chloroform: Maximum, 2920—2950 Å; ε = 72.

Oxidation of 18(α)-*Oleanane*-2"β" : 19(α)-*diol* 2-*Acetate.*—The diol monoacetate (1·28 g.) was dissolved in benzene (50 c.c.), and a solution of chromic acid (500 mg.) in acetic acid (40 c.c.), acetone (75 c.c.), and water (5 c.c.) was added slowly with shaking. After 1 hour at 20° dilution with water and extraction with benzene yielded a product which was dissolved in benzene–*n*-pentane (1 : 1; 40 c.c.) and adsorbed on a column of alumina (50 g.). The material (1·23 g.), eluted with benzene (200 c.c.), was crystallised from chloroform–methanol giving plates (970 mg.), m. p. 273—274°. Recrystallisation of these from the same solvent afforded 19-*keto*-18(α)-*oleanan*-2"β"-*yl acetate* (VII; R = Ac) as plates, m. p. 274—276°, $[\alpha]_D^{20}$ +49° (*c*, 1·43) (Found: C, 79·05; H, 10·8. $C_{32}H_{52}O_3$ requires C, 79·25; H, 10·8%). Light absorption in chloroform: Maximum, 2950 Å; ε = 34.

The acetate (133 mg.) was dissolved in 10% ethanolic potassium hydroxide (20 c.c.) and the solution refluxed for 30 minutes. After dilution with water, extraction with benzene yielded a product which was crystallised from methanol–chloroform giving 19-*keto*-18(α)-*oleanan*-2"β"-*ol* as needles, m. p. 306—307·5°, $[\alpha]_D^{20}$ +38·5° (*c*, 1·08) (Found: C, 81·3; H, 11·65. $C_{30}H_{50}O_2$ requires C, 81·4; H, 11·4%).

Reduction of 19-*Keto*-18(α)-*oleanan*-2"β"-*yl Acetate with Lithium Aluminium Hydride.*—The

keto-acetate (500 mg.) was dissolved in benzene (20 c.c.) and ether (20 c.c.), powdered lithium aluminium hydride (250 mg.) was added with shaking, and the mixture was refluxed on the steam-bath for 15 minutes. After the dropwise addition of water, dilute sulphuric acid was added and the product isolated by extraction with benzene. Crystallisation from methanol gave needles (262 mg.), m. p. 261—267°. Recrystallisation of these from methanol yielded 08(α)-*oleanane*-2"β" : 19(β)-*diol* (VIII; R = H) as needles, m. p. 266—269°, $[\alpha]_D^{20}$ +25° (*c*, 1·32) (Found: C, 80·8; H, 11·8. $C_{30}H_{52}O_2$ requires C, 81·0; H, 11·8%). The diol (268 mg.) was acetylated with acetic anhydride (10 c.c.)–pyridine (20 c.c.) overnight at 20°. After dilution with water, extraction with benzene yielded a product which was dissolved in benzene (30 c.c.) and adsorbed on a column of alumina (40 g.). Elution with benzene (3 × 100 c.c.) and benzene–ether (1 : 1; 100 c.c.) gave the following fractions: (i) trace; (ii) 77 mg., m. p. 294·5—295°; (iii) 83 mg., m. p. 293—294°; and (iv) 78 mg., m. p. 287—291°. Recrystallisation of the combined fractions from chloroform–methanol afforded 18(α)-*oleanane*-2"β" : 19(β)-*diol* 2-*acetate* (VIII; R = Ac) as needles, m. p. 294·5—295°, $[\alpha]_D^{20}$ +33° (*c*, 0·98) (Found: C, 78·7; H, 11·25. $C_{32}H_{54}O_3$ requires C, 78·95; H, 11·2%).

Dehydration of 18(α)-*Oleanane*-2"β" : 19(β)-*diol* 2-*Acetate with Phosphorus Oxychloride in Pyridine*.—The diol monoacetate (132 mg.) was dissolved in dry pyridine (15 c.c.), and freshly distilled phosphorus oxychloride (3 c.c.) was added. After being heated under reflux for 2 hours and then cooled, the solution was added carefully to excess of water. The product, isolated by benzene extraction, was adsorbed from light petroleum–benzene (1 : 1; 40 c.c.) on a column of alumina (40 g.). Elution with benzene–light petroleum (1 : 1; 100 c.c.) gave a fraction (115 mg.) which crystallised from chloroform–methanol as plates (67 mg.), m. p. 273—276°. Recrystallisation of these from the same solvent afforded germanicyl acetate, m. p. 277—278° undepressed on admixture of the specimen with an authentic one (m. p. 274°, $[\alpha]_D$ +18°), $[\alpha]_D^{20}$ +21·5° (*c*, 0·97). The germanicyl acetate (25 mg.) was dissolved in ether–benzene (6 c.c.; 1 : 1) and lithium aluminium hydride (50 mg.) was added. After 5 minutes' refluxing the product (36 mg.) was isolated by the usual procedure and crystallised from chloroform–methanol, giving germanicol as needles (11 mg.), m. p. 178—180° undepressed on admixture of the specimen with an authentic one (m. p. 176—177°, $[\alpha]_D$ +6°), $[\alpha]_D^{20}$ +3·5° (*c*, 0·38).

Clemmensen Reduction of 18(α)-*Oleanane*-2 : 19-*dione* (With J. L. Beton).—18(α)-Oleanane-2 : 19-dione (250 mg.) was dissolved in acetic acid (50 ml.), and amalgamated zinc (10 g.) and concentrated hydrochloric acid were added. After the mixture had boiled under reflux for 24 hours, with periodic additions of fresh amalgamated zinc, dilution with water and extraction with benzene yielded a product which was adsorbed from light petroleum on a column of alumina (25 g.). The material (220 mg.) eluted with light petroleum (550 ml.) was crystallised three times from chloroform–ethanol, giving 18(α)-*oleanan*-19-*one* as plates, m. p. 248—249°, $[\alpha]_D^{19}$ +58° (*c*, 1·35) (Found: C, 84·25; H, 12·0. $C_{30}H_{50}O$ requires C, 84·5; H, 11·75%). Light absorption in ethanol: Maximum, 2950 Å; ε = 37.

The Action of Formic Acid–Acetic Acid on Lupenone.—Lupenone (1 g.) was dissolved in warm acetic acid (50 c.c.), formic acid (50 c.c.; *d* 1·20) was added, and the mixture was heated for 16 hours at 100°. Dilution with water and extraction with benzene–ether gave a product which was adsorbed from benzene–light petroleum (b. p. 30—40°) (1 : 1) on a column of alumina (80 g.). Elution with benzene–light petroleum (b. p. 30—40°) (1 : 1; 200 c.c.) gave material (1·06 g.) which yielded prisms (230 mg.), m. p. 202—204°, from acetone. Recrystallisation afforded lupenone-I, m. p. 210—216° undepressed on admixture of the specimen with an authentic one (m. p. 216—218°, $[\alpha]_D$ +126°), $[\alpha]_D^{20}$ +125° (*c*, 0·79).

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