98. *The Chemistry of the Triterpenes. Part VII.* An Inter-relationship between the Lupeol and the β-Amyrin Series. Elucidation of the Structure of Lupeol.*

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

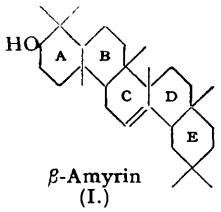
Two of the three major groups of triterpenes have been inter-related. Lupeol derivatives can be isomerised by acid treatment into compounds of the δ-amyrenol series; the relationship of these compounds to the corresponding β-amyrin derivatives, from which they are formed by acid-induced isomerisations, is reasonably well established. Compounds of the β-amyrin and lupeol series are identical in rings A, B, C, and D, except for the presence of the ethylenic bond in ring C in the β-amyrin series and a possible stereochemical difference at $C_{(18)}$, *i.e.*, the junction of rings D and E. If structure (I) is accepted for β-amyrin then it follows, from the results described in this paper, that lupeol must be represented by formula (IV). A preliminary account of this work has already been published (Ames and Jones, *Nature*, 1949, **164**, 1090).

In the last twenty years, largely because of the brilliant and extensive researches of Ruzicka and his school, rigorous relationships have been established between a large majority of the well-characterised pentacylic triterpenoid compounds and one or other of three parent $C_{30}H_{50}O$ alcohols, α-amyrin, β-amyrin, and lupeol. (For summaries, see Spring, *Ann. Reports*, 1941, **38**, 192, and Noller, *Ann. Rev. Biochem.*, 1945, **14**, 383.) Hitherto it has not been possible to effect any interconversions between these parent compounds, but both β-amyrin and lupeol have now been transformed into common intermediates by reactions capable of structural interpretation.

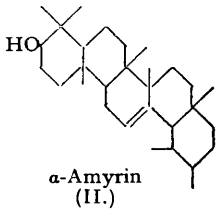
On the basis of a considerable volume of degradative evidence obtained with a number of the triterpenes of the β-amyrin series, the structure (I), originally suggested for β-amyrin by Haworth (*Ann. Reports*, 1937, **34**, 327), has now been established with a considerable degree of certainty (cf. Bischof, Jeger, and Ruzicka, *Helv. Chim. Acta*, 1949, **32**, 1911). For α-amyrin, which is similar in many respects to the β-isomer, the closely related structure (II) has been

* The following are regarded as constituting the earlier members of this series: Part I. Lupanetriol and its Oxidation. *J.*, 1940, 456. Part II. The Oxidation of Lupenyl Esters. *J.*, 1940, 1335. Part III. The Constitution of Lupeol. *J.*, 1941, 757. Part IV. Surface Films of Lupane Derivatives. *J.*, 1941, 761. Part V. Optical-rotatory Power and Structure in Triterpenoid Compounds. Application of the Method of Molecular-rotation Differences. *J.*, 1944, 659. Part VI. The Degradation of Lupeol Derivatives to C_{27}-Ketones. *Rec. Trav. chim.*, 1950, **69**, 368.

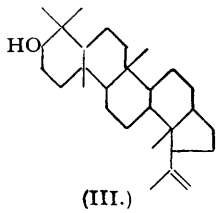
proposed (Meisels, Jeger, and Ruzicka, *Helv. Chim. Acta*, 1949, **32**, 1075), and convincing evidence has recently been adduced (*idem*, *ibid.*, 1950, **33**, 700) concerning the identity in rings



β-Amyrin
(I.)



α-Amyrin
(II.)



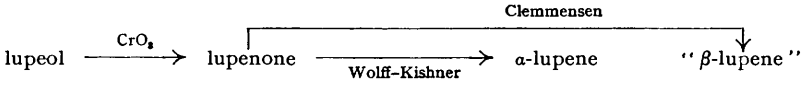
(III.)

A and B of α- and β-amyrin. At first sight it is rather difficult to believe that some of the differences observed between the amyrins arise from the minor structural variation in ring E, but a major stereochemical difference, *i.e.*, at the D—E ring junction, may be involved (cf. Meisels, Jeger, and Ruzicka, *ibid.*, 1950, **33**, 700).

The properties of lupeol and the other members of this group of pentacyclic triterpenes differ sharply in certain respects from those of the α- and the β-amyrins. Thus, in contrast to the latter, lupeol does not afford 1 : 8-dimethylpicene, 1 : 2 : 7-trimethylnaphthalene, or 2 : 7-dimethylnaphthalene on selenium dehydrogenation (Ruzicka, Further, Pieth, and Schellenberg, *Helv. Chim. Acta*, 1937, **50**, 1564) and therefore does not possess a hydropicene carbon-skeleton. Another major differentiation is to be found in the molecular-rotation differences (Barton and Jones, *J.*, 1944, 659) (see Table II). Although it has been shown that the secondary hydroxyl group in lupeol is in a similar environment to that in the amyrin series (Ruzicka, Jeger, and Huber, *Helv. Chim. Acta*, 1945, **28**, 942), the unsaturated centre is quite differently situated, being present in lupeol in a readily hydrogenated *iso*propenyl side-chain (Ruzicka and Rozenkranz, *Helv. Chim. Acta*, 1940, **23**, 1311; Jones and Meakins, *J.*, 1940, 1335). Surface-film measurements (Bilham, Jones, and Meakins, *J.*, 1941, 761) indicate that this *iso*propenyl grouping is attached at the opposite end of the pentacyclic system from the secondary hydroxyl group. The evidence available hitherto has permitted only tentative suggestions to be made concerning the structure of lupeol, with formula (III) finding most favour and providing explanations for the majority of the reactions. The results now to be discussed are best interpreted on a slight modification (IV) of this formula, the angular methyl group being sited at the alternative position at the junction of rings D and E.

Attempts to investigate the structure of ring E of lupeol, following stepwise degradation of the side-chain, have been disappointing (Jones and Meakins, *J.*, 1941, 757; Ruzicka, Huber, and Jeger, *Helv. Chim. Acta*, 1945, **28**, 195; cf. Davy, Jones, and Halsall, *Rec. Trav. chim.*, 1950, **69**, 368). Since only very poor yields of the C_{27} pentacyclic ring ketones could be obtained, it was clear that no really worthwhile progress could be expected from this approach, and that a completely new direction of attack had to be found. It seemed that this might be possible by transforming lupeol or its derivatives into isomeric compounds more susceptible to study by conventional degradative methods.

Some evidence already existed that the double bond in lupeol could migrate under the influence of acidic reagents since Heilbron, Kennedy, and Spring (*J.*, 1938, 329) had found that



lupenone, which gave α-lupene on Wolff–Kishner reduction, yielded an isomeric "β-lupene" on Clemmensen reduction. This isomer, produced only in very poor yield, presumably by acidic isomerisation, was regarded as a pure compound but it now seems that it was possibly an impure specimen of the lupene-I, described below. Further evidence of rearrangement involving the

lupeol ⟶ lupeol hydrochloride ⟶ *iso*lupeol

double bond was provided by Duerden, Heilbron, McMeeking, and Spring (*J.*, 1939, 322), who showed that lupeol could be converted into an *iso*lupeol (obtained as its acetate), by addition of hydrogen chloride to the double bond followed by dehydrochlorination and acetylation.

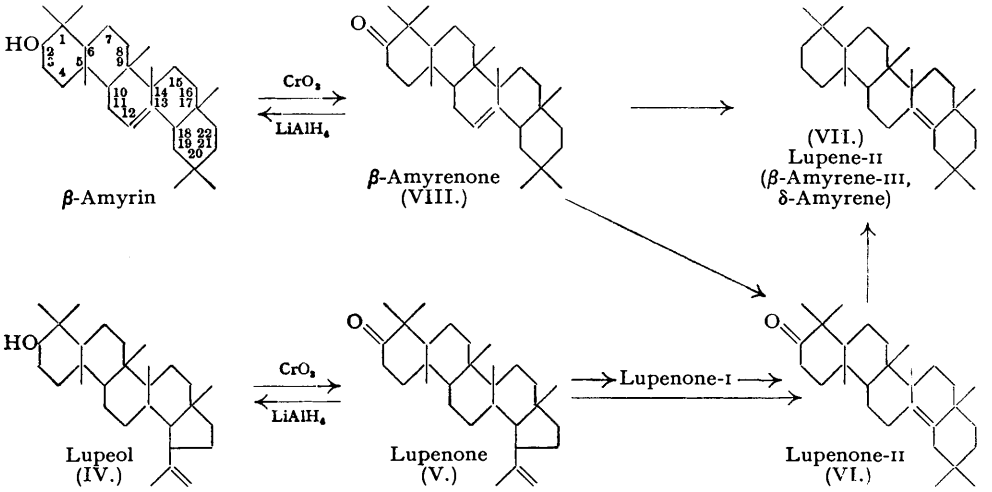
Stimulated by these possibilities, but hardly encouraged by the yields obtained and difficulties encountered by the earlier workers, a systematic study of the influence of acidic

reagents on lupeol derivatives was begun. After a prolonged investigation of the reaction conditions with various lupeol derivatives it has been found possible to convert lupenone (V) into an isomer, lupenone-I, in over 40% yield, by treatment with a solution (*ca.* 6%) of sulphuric acid in acetic acid at room temperature for 4 days. Treatment of lupenone with a solution (*ca.* 15%) of sulphuric acid in benzene–acetic acid at room temperature for 15 days gave a 40% yield of a second isomer, lupenone-II (VI). Lupenone-I has also been converted into lupenone-II under the same conditions. Concentration of acid, rather than time of reaction, appears to determine whether lupenone-I or lupenene-II is formed in the isomerisation reaction.

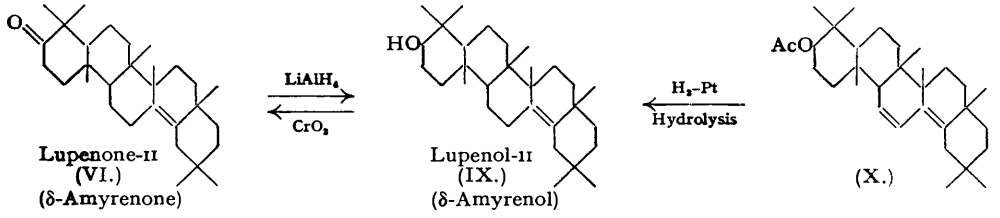
Reduction of lupenone-II by the Wolff–Kishner procedure gave a hydrocarbon, lupene-II (VII). This compound has been shown to be identical with β-amyrene-III (δ-amyrene; see later), prepared from β-amyrenone (VIII) by reduction with amalgamated zinc and hydrochloric acid according to Winterstein and Stein (*Annalen*, 1933, **502**, 223). The constants for the products obtained by the alternative routes are compared in Table I; no melting-point depression was observed with a mixture of lupene-II and β-amyrene-III, and their infra-red spectra, determined in suspension in "Nujol," were indistinguishable.

Isomerisation of β-amyrenone (VIII) in benzene solution with sulphuric acid in acetic acid also gave lupenone-II (VI) (δ-amyrenone—see later), whose melting point was undepressed on admixture with lupenone-II prepared from lupenone. Identical 2 : 4-dinitrophenylhydrazones were obtained from the ketones prepared by the two routes (cf. Table I).

Reduction of lupenone-II with lithium aluminium hydride gave an alcohol, lupenol-II (IX), with physical constants identical with those of δ-amyrenol (see Table I) (Ruzicka and Jeger,



Helv. Chim. Acta, 1941, **24**, 1243; Ruzicka, Jeger, and Norymberski, *Helv. Chim. Acta*, 1942, **25**, 457), the structure of which seems to be indicated from its preparation from the dienyl acetate (X) (cf. Barton and Brooks, *J. Amer. Chem. Soc.*, 1950, **72**, 3314) by partial hydrogenation and hydrolysis. The melting point of δ-amyrenol was not depressed by



admixture with lupenol-II. Comparisons were also made between the corresponding acetates and benzoates. Oxidation of δ-amyrenol with chromic acid gave δ-amyrenone, which, as already indicated, had the same physical constants as, and gave no depression in melting point on admixture with, lupenone-II (see Table I).

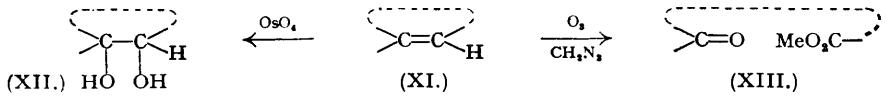
TABLE I.

	Observed. M. p.	Observed. $[\alpha]_D^{20}$.	Literature. M. p.	Literature. $[\alpha]_D^{20}$.
(1) Lupenone-II (from lupenone)	199·5—202·5°	−12°	—	—
(2) Lupenone-II (from β-amyrenone)	200 —202·5	−12	—	—
(3) δ-Amyrenone (from δ-amyrenol)	199·5—201	−12	—	—
2 : 4-Dinitrophenylhydrazone of (1)	222 —224	+22	—	—
2 : 4-Dinitrophenylhydrazone of (2)	222 —223	+19	—	—
2 : 4-Dinitrophenylhydrazone of (3)	223 —224	+21	—	—
(4) Lupene-II (from lupenone)	191·5—192	−33·5	—	—
(5) β-Amyrene-III (from β-amyrenone) [1]	191·5—192·5	−32·5	187 —189·5°	−22°
(6) Lupenol-II (from lupenone)	212 —212·5	−50·5	—	—
(7) δ-Amyrenol (from β-amyrin) [2]	211·5—213·5	−49·5	213 —213·5	−52
(8) Acetate of (6)	207·5—209	−34	—	—
(9) Acetate of (7) [2]	208·5—209·5	−34	208·5—209·5	−35
(10) Benzoate of (6)	225 —226·5	− 7·5	—	—
(11) Benzoate of (7) [2]	225 —227	—	224 —225	− 8

[1] Winterstein and Stein, *loc. cit.* [2] Ruzicka and Jeger, *loc. cit.*

Since the identities of δ-amyrenol and lupenol-II, and δ-amyrenone and lupenone-II, have been established and since these compounds contain the β-amyrin carbon-skeleton, the amyrin nomenclature is obviously to be preferred to that based on lupenone-II, but both nomenclatures are used in this paper in order to indicate the source of the compound under discussion. As has already been indicated, the structure of δ-amyrenol (lupenol-II) can be regarded as established by the work of Ruzicka and Jeger (*loc. cit.*). The oxidation of δ-amyrenol to δ-amyrenone by chromic acid leads to structure (VI) for lupenone-II (δ-amyrenone) provided no isomerisation of the double bond occurred during the oxidation. That this is so follows from the reduction of δ-amyrenone back to δ-amyrenol by lithium aluminium hydride, wherein no rearrangement of the double bond would be expected to occur (cf. Shoppee and Summers, *J.*, 1950, 687; Koller, Dietrich, and Jeger, *Helv. Chim. Acta*, 1950, **33**, 1050). The major products of the reduction of β-amyrenone and lupenone with this reagent are β-amyrin and lupeol, respectively. These results indicate that the hydroxyl group at $C_{(2)}$ in β-amyrin has the same configuration as that at $C_{(2)}$ in δ-amyrenol and in lupeol. From formula (VI) for lupenone-II, structure (VII) follows for lupene-II (β-amyrene-III), it being generally understood that ethylenic-bond migrations do not occur on Wolff–Kishner reduction other than in αβ-unsaturated carbonyl compounds. The results described in the following paper provide additional confirmation that no rearrangements occur during this reduction. Structure (VII) receives confirmation from the infra-red spectrum of lupene-II, which is compatible with the presence of a tetra-substituted double bond, which can only be present in the **13** : 18-position.

Wolff–Kishner reduction of lupenone-I gives lupene-I, which is also obtained by the direct isomerisation of α-lupene with sulphuric acid in acetic acid–benzene or ethanol. Lupene-I can also be prepared directly from lupenone (V) by reduction under Clemmensen conditions. The infra-red spectrum of this new hydrocarbon (partial formula XI) shows a strong band at 800 cm.$^{-1}$, suggestive of the presence of a trisubstituted double bond. A similar band appears in the spectra of α-pinene, Δ^5-cholestene, and analogously substituted olefins. Lupene-I is



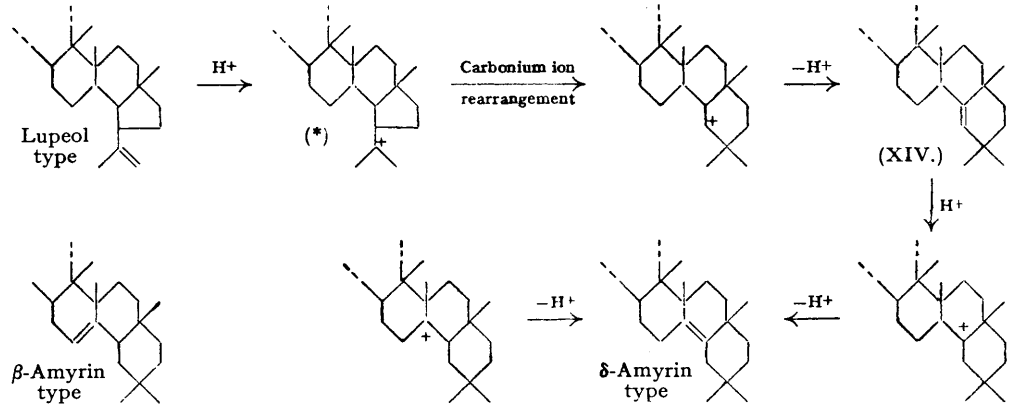
(XII.) (XI.) (XIII.)

converted by treatment with osmic acid into a diol (XII), which forms only a monoacetate. Ozonolysis of lupene-I gave no significant amounts of low molecular-weight carbonyl compounds, indicating that the ethylenic bond is in a ring, and the acidic fraction of the ozonolysis product gave, after esterification with diazomethane, a keto-ester, $C_{31}H_{52}O_3$ (XIII). This did not form ketonic derivatives, but the presence of the keto-group was clearly indicated by its absorption in the ultra-violet region.

Reduction of lupenone-I with lithium aluminium hydride yielded an alcohol, lupenol-I, from which the acetate and benzoate were prepared. It was found possible to obtain lupenyl-I acetate directly from lupenyl acetate, albeit in poor yield, by isomerisation of the latter in benzene solution with sulphuric acid in glacial acetic acid. The poor yield was not unexpected

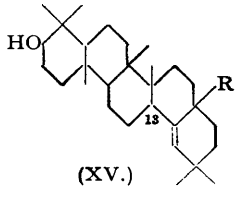
as we have found that the acetoxy-group of triterpene acetates is rather unstable under the acidic conditions used for the isomerisations described. Attempts to isomerise lupenyl-I acetate or lupenyl acetate directly to δ-amyrenyl acetate were not very successful but in one experiment δ-amyrenyl acetate was isolated from the isomerisation products resulting from lupenyl acetate. This result is significant because it provides additional evidence that the hydroxyl group at $C_{(2)}$ in the lupeol series has the same configuration as that at $C_{(2)}$ in the β-amyrin series. This identity of configuration has already been indicated by the lithium aluminium hydride reduction of β-amyrenone and lupenone to β-amyrin and lupeol, respectively.

At the present time it is not possible to put forward unique structures for the compounds of the lupene-I series. Structure (XIV) is not inconsistent with the evidence given above, and it fits conveniently into the picture as an intermediate in the mechanism indicated below for the conversion from the lupeol series into the δ-amyrin series. This mechanism involves rearrangements which are already familiar in terpene and steroid chemistry. However, recent work by Barton and Brooks (*loc. cit.*) has provided convincing evidence that structure (XIV) must be ascribed to the triterpene germanicol (full structure, XV ; R = Me) (Simpson, *J.*, 1944, 283). Lupenol-I and germanicol are not identical, and hence structure (XIV) could not represent the lupene-I series unless lupenol-I and germanicol are stereoisomeric at $C_{(13)}$. That this is not the case has now been shown by Davy, Halsall, and Jones, *Chem. and Ind.*, 1950, 732) by the conversion of betulin (XVI), a member of the lupeol group which must have the same configuration at $C_{(13)}$ as lupenol-I, into moradiol (XV; R = CH_2·OH) (Barton and Brooks, *loc. cit.*) belonging to the germanicol group. If the above mechanism is correct, germanicol derivatives should be isomerised by acidic reagents into compounds of the lupene-II type. Since no germanicol derivatives have yet been isolated from the products of the isomerisation of lupenone to lupenone-II, it is believed that such derivatives probably isomerise under less



drastic conditions than those necessary for the conversion of lupenone into lupenone-II. Indeed some evidence of this possibility in a more complicated case has been provided by David (cf. *Bull. Soc. chim.*, 1950, **17**, 169). This point is under investigation.

It is believed that the lupene-I type skeleton is formed as a result of the carbonium ion (*) being capable of undergoing other transformations in addition to those postulated in the above mechanism, that these other transformations lead to lupene-I and its derivatives, and that,



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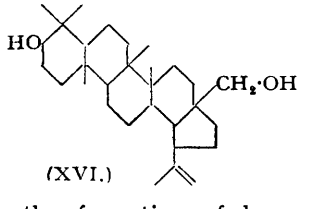
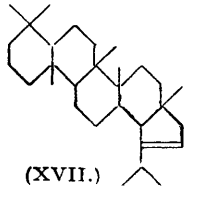


(XVI.)


(XVII.)

under suitable conditions, as in the formation of lupenone-II from lupenone-I, these transformations are reversible. Further work on the structure of lupene-I and its derivatives is in progress. One of the structures being considered is (XVII).

These results lead to the conclusion that the structures of rings A, B, C, and D of lupeol and

β-amyrin are identical apart from the presence of the double bond at the 12 : 13-position in β-amyrin and a possible difference in stereochemical configuration at $C_{(18)}$. They further indicate that, if β-amyrin has structure (I), then lupeol has the structure (IV), since it is only possible to formulate a reasonable mechanism for the formation of δ-amyrin derivatives if structure (IV) is correct. Various other structures which have been suggested for lupeol would give rise to compounds other than δ-amyrin derivatives on isomerisation.

TABLE II.

	Alcohol	Acetate (1)	Benzoate (2)	Ketone (3)	Δ_1	Δ_2	Δ_3
α- and β-Amyrin [1] group	—	—	—	—	+ 6	+145	+ 60
Lupeol–betulin [1] group ...	—	—	—	—	+70	+200	+140
Lupenol-I	+400	+444	+572	+534	+44	+172	+134
δ-Amyrenol [2]	−216	−161	− 41	− 51	+55	+175	+165

[1] Barton and Jones, *loc. cit.*

[2] The figures given are the average of those determined in the present study and by Ruzicka and Jeger (*loc. cit.*).

Although it would not be wise to attach much significance to the values at this stage, the molecular-rotation differences associated with lupenol-I and δ-amyrenol are listed in Table II, where they are compared with those of the α- and β-amyrin and lupeol groups.

EXPERIMENTAL.

(All melting points were determined on a Kofler block and are corrected, unless specifically stated. Rotations were determined in chloroform. Light petroleum refers to the fraction with b. p. 40—60° unless otherwise stated.)

*Lupene-*I.—(a) *Isomerisation of α-lupene.* (i) α-Lupene (1 g.) in benzene (15 c.c.) was treated at room temperature with a mixture of acetic acid (45 c.c.) and sulphuric acid (3·5 c.c.; *d* 1·84). The solution rapidly became brown, and the temperature rose to 40°, thus preventing immediate precipitation of α-lupene. After 5 days at 20°, dilution with water and isolation with ether yielded a brown resin (1·13 g.). This was dissolved in benzene (10 c.c.)–light petroleum (10 c.c.) and adsorbed on to a column of alumina (50 g.; activity I—II). Elution with light petroleum (400 c.c.) afforded *lupene-*I, crystallising from ethyl acetate as platelets (360 mg.; 36%), m. p. 208—210°, $[\alpha]_D^{20}$ +82° (*c*, 1·20). Several recrystallisations from ethyl acetate and from chloroform–methanol raised the m. p. to 224·5—225·5°, $[\alpha]_D^{20}$ +102° (*c*, 1·10) (Found : C, 87·5; H, 11·9. $C_{30}H_{50}$ requires C, 87·75; H, 12·25%). In the Liebermann–Burchard test, a deep-red colour was obtained; with tetranitromethane, a yellow colour.

(ii) α-Lupene (3 g.) in benzene (75 c.c.) was refluxed with ethanolic sulphuric acid (375 c.c.; 15%) for 48 hours. After cooling to 0°, the separated solid was purified by chromatography and crystallised from ethyl acetate, giving platelets, m. p. 213—214° (210 mg.; 7%). The melting point was raised by several recrystallisations from ethyl acetate and chloroform–methanol to 223—224°, undepressed on admixture with lupene-I prepared by method (b).

(b) *Clemmensen reduction of lupenone.* Amalgamated zinc (60 g.) was covered with a solution of lupenone (8 g.) in acetic acid (300 c.c.) and water (40 c.c.). The solution was refluxed with concentrated hydrochloric acid (55 c.c.), a further five portions (30 c.c. each) being added over a period of 8½ hours. The solution was diluted with water, and the product was isolated by repeated ethereal extraction. Evaporation of the washed and dried extract, followed by careful addition of methanol, afforded lupene-I (770 mg.; 10%), m. p. 200—203° (capillary), $[\alpha]_D$ +86° (*c*, 1·25). The melting point was raised to 223—224° by several recrystallisations from ethyl acetate and chloroform–methanol.

*Lupenone-*I.—Lupenone (3 g.) in acetic acid (50 c.c.) was treated with a mixture of acetic acid (10 c.c.) and sulphuric acid (5 c.c.; *d* 1·84). The solution was kept at 20° for 96 hours, by which time it had developed a green fluorescence and deposited clusters of needles. These were recrystallised from chloroform–methanol giving needles of *lupenone-*I (1·49 g.; 50%), m. p. 187—192°, raised to 217·5—218·5° by several recrystallisations from chloroform–methanol; $[\alpha]_D^{20}$ +126° (*c*, 2·82) (Found : C, 84·75; H, 11·35. $C_{30}H_{48}O$ requires C, 84·8; H, 11·4%). Light absorption in chloroform–ethanol (1 : 4) : Maximum, 2860—2920 A.; ε = 30·5. The Leibermann–Burchard reagent gave a brown colour. More lupenone-I (*ca.* 15% yield) was isolated by dilution and ethereal extraction of the residual reaction mixture, followed by chromatographic purification of the solid obtained from the ether extract.

*Lupenone-*I 2 : 4-*dinitrophenylhydrazone* crystallised from ethanol–benzene as orange needles, m. p. 266—267° (decomp.) (Found : N, 9·2. $C_{36}H_{52}O_4N_4$ requires N, 9·3%).

*Lupenone-*II.—(*a*) Lupenone (5 g.) in benzene (50 c.c.) was treated with a mixture of glacial acetic acid (425 c.c.) and sulphuric acid (75 c.c.; *d* 1·84). The solution was warmed to 50° and then kept at 20° for 15 days. The resultant brown solution (green fluorescence) was poured into water, and the resinous product, isolated with ether, was dissolved in benzene (20 c.c.), adsorbed on a column of alumina (350 g.; activity I—II), and eluted first with light petroleum (750 c.c.), and then with light petroleum–benzene (1 : 1) (900 c.c.). The material (2·81 g.) obtained from this second eluate was recrystallised from ethanol–acetone (1 : 1) yielding *lupenone-*II as platelets (2·1 g.; 42%), m. p. 181—188° raised by many recrystallisations from acetone–methanol and chloroform–methanol to 199·5—202·5°, $[\alpha]_D^{20}$ −12° (*c*, 1·05) (Found : C, 84·8; H, 11·7. $C_{30}H_{48}O$ requires C, 84·8; H, 11·4%). Light absorption in chloroform–ethanol (1 : 4) : Maxima, 2430, 2510, 2620 (inflexion), and 2870—2920 A.; ε = 1000,

1000, 1000 (all approx.), and 33, respectively. The band at 2870—2920 A. shows the presence of a ketonic grouping. The bands at 2430, 2510, and 2620 A. indicate the presence of a small amount (*ca.* 3—4%) of a diene impurity, probably the ketone corresponding to β-amyradienol-II which shows these same maxima (Green, Mower, Picard, and Spring, *J.*, 1944, 527). In the Liebermann–Burchard test a deep reddish-purple colour was obtained. The behaviour of lupenone-II on the Kofler block was very characteristic, as it partly melted and then assumed a new crystal form (needles) at 170—190° before melting finally at the temperature given. *Lupenone*-II 2 : 4-*dinitrophenylhydrazone* crystallised from chloroform–methanol as platelets, m. p. 222—224°, $[\alpha]_D^{20}$ +22° (*c*, 0·98) (Found : N, 9·4. $C_{36}H_{52}O_4N_4$ requires N, 9·3%). Light absorption in ethanol : Maximum, 3700—3720 A.; ε = 25,000.

(*b*) Lupenone-I (500 mg.) was treated exactly as for the preparation of lupenone-II from lupenone. The resinous product obtained after 15 days' storage at 20° was adsorbed on a column of alumina (70 g.; activity I—II) and fractionally eluted with benzene–light petroleum (1 : 1). A fraction (0·35 g.) was obtained which crystallised from acetone–ethanol as platelets, m. p. 199—202° undepressed on admixture with a specimen of lupenone-II prepared from lupenone; it had $[\alpha]_D^{20}$ −2·5° ± 5° (*c*, 0·237). Insufficient material prevented further purification.

(*c*) β-Amyrenone (1·3 g.) in benzene (13 c.c.) was treated with acetic acid (110 c.c.) and sulphuric acid (19·5 c.c.; *d* 1·84). The solution was warmed to 80° and then kept at 20° for 14 days. The resultant reddish-brown solution was worked up as in method (*a*) above, except that benzene was used as the extractant in order to avoid emulsification. Elution of the product from alumina (100 g.; reactivated; activity I) with light petroleum–benzene (2 : 3) (500 c.c.) gave a partially crystalline product (981 mg.) which, after several recrystallisations from ethanol and acetone–methanol, gave lupenone-II as flat needles, m. p. 200—202·5° undepressed on admixture with a specimen prepared from lupenone by the method described above; it had $[\alpha]_D^{20}$ −12° (*c*, 1·39). The 2 : 4-dinitrophenylhydrazone, which crystallised as platelets from chloroform–methanol, had m. p. 222—223°, undepressed on admixture with the 2 : 4-dinitrophenylhydrazone described above, $[\alpha]_D^{20}$ +19° (*c*, 0·42).

Hydrogenation of Lupene-I.—Lupene-I (16·3 mg.) in ethyl acetate–acetic acid (1 : 1) (10 c.c.) was shaken in a microhydrogenation apparatus with pre-reduced Adams's platinum catalyst (16·1 mg.) and hydrogen for 44 hours at 20° (Found : uptake of hydrogen, 0·87 c.c. Required for 1 double bond : 0·89 c.c.) The saturated *hydrocarbon* formed crystallised from ethyl acetate as platelets, m. p. 232—233°, $[\alpha]_D^{20}$ +26° (*c*, 0·57) (Found : C, 87·6; H, 12·35. $C_{30}H_{52}$ requires C, 87·3; H, 12·7%). The hydrocarbon gave no colour with tetranitromethane.

Hydroxylation of Lupene-I.—Lupene-I (770 mg.) in dry pyridine (21 c.c.) and dry chloroform (25 c.c.) was treated with osmic acid (470 mg.), and the solution was kept at 20° for 26 days. The resulting dark solution was evaporated to dryness under reduced pressure at 60°, and the residue was refluxed with mannitol (3 g.) in ethanol (20 c.c.), benzene (10 c.c.), and sodium hydroxide solution (10 c.c.; 2N.) for 10 hours. Dilution with water and isolation with benzene yielded a solid which was fractionated on a column of alumina (activity I—II). A fraction, eluted with ether, afforded a *diol* (474 mg.; 62%) crystallising from ethyl acetate as platelets, m. p. 288—291°, $[\alpha]_D^{20}$ +12° (*c*, 1·79) (Found : C, 80·75; H, 11·55. $C_{30}H_{52}O_2$ requires C, 81·0; H, 11·8%). The diol (130 mg.) was acetylated in dry pyridine (3 c.c.) at 20° with acetic anhydride (1 c.c.) for 20 hours. The product (120 mg.) gave, on crystallisation from methanol, platelets of a *monoacetate*, m. p. 264—265·5°, $[\alpha]_D^{20}$ −8° (*c*, 1·97) (Found : C, 78·7; H, 10·9. $C_{32}H_{54}O_3$ requires C, 78·95; H, 11·2%).

Wolff–Kishner Reduction of Lupenone-I.—Lupenone-I (1·2 g.) was heated in an autoclave for 5 hours at 175° and 100 atmospheres with hydrazine hydrate (7·8 c.c.; 60%) and a solution of sodium (1·5 g.) in ethanol (30 c.c.). After the reduction the mixture was diluted with water, and the precipitated solid was isolated; it crystallised from ethyl acetate as platelets (0·9 g.; 75%), m. p. 222—223° undepressed on admixture with a specimen of lupene-I prepared by the isomerisation of α-lupene.

Wolff–Kishner Reduction of Lupenone-II.—Lupenone-II (720 mg.) was heated in an autoclave for 7 hours at 200° and 50 atmospheres with hydrazine hydrate (2 c.c.; 60%) and a solution of sodium (0·88 g.) in ethanol (18·7 c.c.). The product crystallised from methanol–chloroform as platelets (552 mg.; 77%), m. p. 191·5—192°, $[\alpha]_D^{20}$ −33·5° (*c*, 1·11). The m. p. was undepressed on admixture with a specimen of β-amyrene-III prepared from β-amyrenone by Winterstein and Stein's method (*loc. cit.*). The infra-red spectra of lupene-II and β-amyrene-III were identical.

Ozonolysis of Lupenone-I.—Lupenone-I (1·0 g.) in acetic acid (50 c.c.) (redistilled from chromic acid) was treated with a stream of ozonised oxygen (6%) for 3 hours (≡ 4·5 mol. of O_3), the exit gases being passed through two wash-bottles containing water. The reaction mixture was then steam-distilled until 400 c.c. of distillate had been collected. The formaldehyde content of the combined distillate and washings was estimated as formaldehyde dimedone, m. p. 186—187° undepressed on admixture with an authentic specimen (yield of the dimedone : 16·6 mg., 2·4%). Lupenone under comparative conditions gave 26 and 22% yields of formaldehyde.

Ozonolysis of Lupene-I.—Lupene-I (1 g.) in chloroform (100 c.c.) was treated with a stream of ozonised oxygen (6%) for 5½ hours at 10°. The chloroform was removed at 0° and the residue steam-distilled. The distillate, together with the aqueous washings of the ozoniser exit gases, was treated with 2 : 4-dinitrophenylhydrazine sulphate solution. No precipitate resulted. The residue from the steam-distillation gave an acidic fraction (0·79 g.) which was esterified with diazomethane in ethereal solution. The crude ester was chromatographed on alumina (70 g.; reactivated; activity I); elution with benzene gave the *keto-ester* (0·45 g.; 39%), which crystallised from methanol as needles, m. p. 184—186°, $[\alpha]_D$ +17° (*c*, 0·42) (Found : C, 78·95; H, 10·7. $C_{31}H_{52}O_3$ requires C, 78·75; H, 11·1%). Light absorption in chloroform–ethanol (1 : 4) : Maximum, 2700—2790 A.; ε = 37·5. The ester gave no colour with the Liebermann–Burchard reagent, nor did it form a 2 : 4-dinitrophenylhydrazone under the normal conditions.

Reduction of β-Amyrenone.—β-Amyrenone (500 mg.) in anhydrous tetrahydrofuran (30 c.c.) was treated at 20° with a solution of lithium aluminium hydride in dry ether (2·85 c.c.; 0·375M.). Next morning the complex was decomposed with excess of sulphuric acid (2N.) with ice-cooling. Further dilution with water, followed by isolation of the product in the usual manner, and crystallisation from chloroform–methanol gave needles of β-amyrin (273 mg.; 55%), m. p. 197—199° undepressed on admixture with an authentic specimen, $[\alpha]_D$ +91° (*c*, 1·23). (Literature values for β-amyrin: m. p. 197—197·5°, $[\alpha]_D^{20}$ +88·4°.)

Reduction of Lupenone.—Lupenone (350 mg.) was reduced with lithium aluminium hydride, as above, giving lupeol, which crystallised from acetone as needles (210 mg.; 60%), m. p. 209—212°, $[\alpha]_D$ +33° (*c*, 0·41). Three recrystallisations from acetone raised the m. p. to 214—215°, undepressed on admixture with an authentic specimen, $[\alpha]_D$ +28·5° (*c*, 2·15). [Literature values for lupeol: m. p. 215—216°, $[\alpha]_D^{20}$ +26·4° (*c*, 3·51).]

*Reduction of Lupenone-*I.—Lupenone-I (365 mg.) was reduced with lithium aluminium hydride as above, giving *lupenol-*I, crystallising from chloroform–methanol as platelets (219 mg.; 60%), m. p. 253—254°, $[\alpha]_D$ +94° (*c*, 1·37) (Found: C, 84·25; H, 11·7. $C_{30}H_{50}O$ requires C, 84·4; H, 11·8%). Acetylation gave *lupenyl-*I *acetate*, which separated from methanol–chloroform as platelets, m. p. 243—244°, $[\alpha]_D^{20}$ +95° (*c*, 1·01) (Found: C, 82·05; H, 11·0. $C_{32}H_{52}O_2$ requires C, 82·0; H, 11·2%). Benzoylation of lupenol-I with benzoyl chloride in pyridine gave *lupenyl-*I *benzoate*, which crystallised from methanol–chloroform as platelets, m. p. 273—275°, $[\alpha]_D^{20}$ +108° (*c*, 1·375) (Found: C, 83·65; H, 10·5. $C_{37}H_{54}O_2$ requires C, 83·7; H, 10·25%).

*Reduction of Lupenone-*II.—Lupenone-II (500 mg.) was reduced with lithium aluminium hydride as above, giving lupenol-II, which crystallised from methanol as needles (368 mg.; 74%), m. p. 212—212·5° undepressed on admixture with δ-amyrenol prepared from β-amyradienol-II by Ruzicka and Jeger's method (*loc. cit.*), $[\alpha]_D^{20}$ −50·5° (*c*, 2·00). Acetylation of lupenol-II gave lupenyl-II acetate, which crystallised from chloroform–methanol as platelets, m. p. 207·5°—209° undepressed on admixture with δ-amyrenyl acetate, $[\alpha]_D^{20}$ −34° (*c*, 0·37). Benzoylation of lupenol-II with benzoyl chloride in pyridine gave lupenol-II benzoate, which crystallised from chloroform–methanol as platelets, m. p. 225—226·5° undepressed on admixture with δ-amyrenyl benzoate, $[\alpha]_D^{20}$ −7·5° (*c*, 0·65).

δ-Amyrenone.—δ-Amyrenol (200 mg.), prepared from β-amyrin, was dissolved in acetic acid (40 c.c.)–chloroform (5 c.c.) and treated at 20° with a solution of chromic acid (70 mg.) in 95% acetic acid (5 c.c.). The mixture was kept for 16 hours, and the product was then isolated by dilution of the solution with water, and ethereal extraction. Crystallisation from chloroform–methanol gave platelets (25 mg.; 63%), m. p. 197—199°, $[\alpha]_D$ −9° (*c*, 1·03). Repeated recrystallisation from acetone–methanol gave platelets of δ-amyrenone, m. p. 199·5—201° undepressed on admixture with lupenone-II, $[\alpha]_D^{20}$ −12° (*c*, 1·06). The δ-amyrenone showed the characteristic melting-point behaviour of lupenone-II. Light absorption in chloroform–ethanol (1 : 4): Maxima, 2510, 2625 (inflexion), and 2900 A.; ε = 500, 400, and 30, respectively. As in the case of lupenone-II derived from lupenone the spectrum shows the existence of a ketonic grouping and indicates the presence of a small amount (*ca.* 1—2%) of a diene impurity. δ-Amyrenone 2 : 4-dinitrophenylhydrazone crystallised from chloroform–methanol as platelets, m. p. 223—224° undepressed on admixture with lupenone-II 2 : 4-dinitrophenylhydrazone, $[\alpha]_D^{20}$ +21° (*c*, 1·12). Light absorption in ethanol: Maximum, 3700—3720 A., ε = 25,000.

Isomerisation of Lupenyl Acetate.—(*a*) Lupenyl acetate (1·5 g.) in benzene (30 c.c.) was treated with a mixture of concentrated sulphuric acid (12 c.c.) and acetic acid (120 c.c.), and the solution was kept at 20° for 40 hours. It was then diluted with water, and a yellow solid was isolated by ethereal extraction. This was dissolved in benzene (10 c.c.) and adsorbed on a column of alumina (150 g.; activity I—II). Elution with light petroleum–benzene (1 : 1) (500 c.c.) gave a solid (800 mg.) which was crystallised from acetone–methanol as platelets, m. p. 227—230° raised by several further recrystallisations (with heavy loss of material) to 244—245° and then undepressed with an authentic specimen of lupenyl-I acetate prepared from lupenone-I. It had $[\alpha]_D^{20}$ +93° (*c*, 1·65).

(*b*) (With Mr. G. S. Davy). Lupenyl acetate (5 g.) was dissolved in benzene (25 c.c.) and a mixture of acetic acid (350 c.c.) and concentrated sulphuric acid (80 c.c.) was added. After 8 days at 20° about one-third of the solution (175 c.c.) was worked up, excess of water being added, and the aqueous solution extracted with benzene. The extract yielded a solid (0·92 g.) which was dissolved in benzene–pentane (1 : 1) and adsorbed on a column of alumina (60 g.; activity I). From the fifth 100-c.c. fraction eluted with benzene–light petroleum (1 : 1), a solid (60 mg., $[\alpha]_D^{20}$ +14°, was obtained. This was recrystallised twice from chloroform–methanol, giving flat needles, m. p. 208—208·5°, $[\alpha]_D^{20}$ −20° (*c*, 0·5). A mixture with authentic δ-amyrenyl acetate, m. p. 209—209·5°, melted at 208—208·5°. These data indicate that the solid is essentially δ-amyrenyl acetate.

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