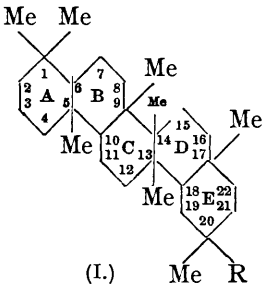
## 246. *The Triterpene Group. Part II. Observations on the Carbon Skeleton of the Triterpenes.*

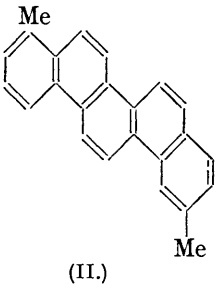
By JAMES C. E. SIMPSON.

The compound $C_{30}H_{44}OS$ obtained by mild dehydrogenation of β-amyrin with sulphur (Jacobs and Fleck, *J. Biol. Chem.*, 1930, **88**, 137) is shown to contain a benzenoid ring which is formed without loss of carbon atoms. This observation is irreconcilable with any skeleton formula hitherto advanced for the oleanolic acid group of triterpenes. The relationship between the thio-compound and two oxidation products derived from it (Jacobs and Fleck, *loc. cit.*) is elucidated.

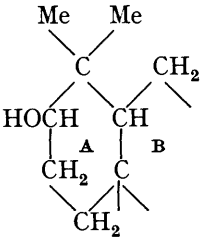
THE present status of triterpene chemistry finds expression in the structure (I, R = H), which represents the carbon skeleton of such triterpenes as yield 1 : 8-dimethylpicene (II) on dehydrogenation with selenium (Ruzicka, Goldberg, and Hofmann, *Helv. Chim. Acta*, 1937, **20**, 325). This structure has been derived by combining the "isoprene hypothesis" with (*a*) data obtained by the selenium dehydrogenation method and (*b*)




(I.)




(II.)




(III.)

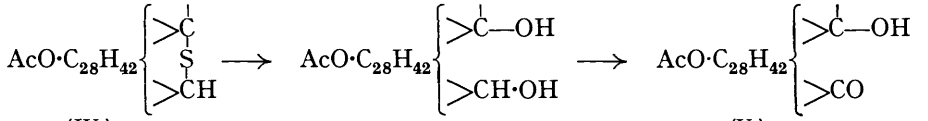
evidence arising from degradations of the triterpene molecule carried out by various investigators, notably by Jacobs, Kitasato, and their co-workers. Whereas this latter evidence, so far as concerns the actual carbon skeleton, has been furnished solely by scission of rings A and B, with the result that the structure of these two rings is known with certainty to the extent represented by the partial structure (III) (for a summary of this evidence see Ruzicka, Hofmann, and Schellenberg, *Helv. Chim. Acta*, **1936**, **19**, 1391), yet the only chemical evidence relative to the nature of rings C—E, on the other hand, is the formation of sapotalin, 2 : 7-dimethylnaphthalene, and 1 : 8-dimethylpicene during dehydrogenation. It is conceivable that these hydrocarbons might originate from a 1 : 8-dimethylperhydropicene skeleton in which rings C—E carry methyl groups in positions other than those shown in (I, R = H), and in view of the known fallibility of the isoprene

rule in sterol chemistry the possibility of ascertaining the detailed structure of rings C—E by other methods merits examination.

In this paper are described certain results bearing on this question which have been obtained by an extension of some earlier observations of Jacobs and Fleck. These authors showed (*J. Biol. Chem.*, 1930, **88**, 137, 153; 1932, **96**, 341) that β-amyrin, hedraganic methyl ester, and oleanolic methyl ester, when heated with sulphur to about 230°, are converted into compounds in which, although six atoms of hydrogen have been removed with simultaneous introduction of one atom of sulphur, the entire triterpene skeleton has apparently been preserved. The sulphur atom was shown to be cyclic in nature by its stability towards lead acetate and alcoholic potash under reflux, but on treatment with permanganate at room temperature it was removed and two well-defined oxidation products were obtained. Thus treatment of β-amyrin, $C_{30}H_{50}O$, with sulphur furnished an alcohol, $C_{30}H_{44}OS$, oxidation of which (as the benzoate) produced a ketone, $C_{30}H_{46}O_3$, and a lactone, $C_{30}H_{44}O_4$; the thio-compounds derived from hedraganic methyl ester and oleanolic methyl ester behaved analogously. The formation of these oxidation products, which are relatively simple derivatives of the parent thio-compounds, indicates the remarkable stability of that portion of the molecule from which six hydrogen atoms were removed during the production of the latter compounds. The aromatisation of a hydroaromatic ring would provide a reasonable explanation of this stability, and an investigation of the structure of the alcohol $C_{30}H_{44}OS$ from β-amyrin has accordingly been commenced in this laboratory.

It appeared in the first place essential to determine accurately the molecular weight of this alcohol, because the analytical figures of Jacobs and Fleck (*loc. cit.*), although in good agreement with their suggested formula, do not definitely exclude the possibility that one, or even two, methyl groups may have been eliminated during the dehydrogenation. For this purpose the *acetate*, m. p. 197—198°, of the thio-compound was prepared and quantitatively saponified according to the method of Sandqvist and Gorton (*Ber.*, 1930, **63**, 1935); the results obtained (see Experimental) clearly indicate that the whole of the original carbon skeleton is present in the thio-compound.

The above-mentioned acetate was also employed, on account of its greater solubility in acetic acid, for the preparation of the $C_{30}$-ketone by permanganate oxidation. The ketone so obtained was invariably purer than the sample of Jacobs and Fleck, and gave analytical data indicating the formula $C_{30}H_{44}O_3$ in contrast to the formula $C_{30}H_{46}O_3$ favoured by the American workers. (A formula analogous to $C_{30}H_{46}O_3$ was also advanced by these authors for the ketonic compound from hedraganic methyl ester; their suggested formula for the corresponding substance from oleanolic methyl ester, however, contains two hydrogen atoms relatively less, and therefore agrees with the formula advocated in this paper.) The formula $C_{30}H_{44}O_3$ admits of a simple explanation of the formation of the ketone (V) (as acetate) from the acetate $C_{32}H_{46}O_2S$ (IV):



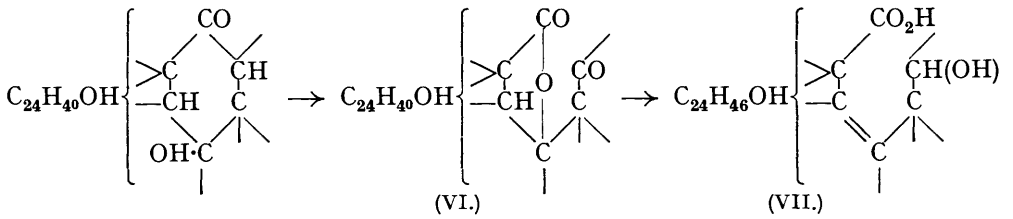

(IV.) (V.)

A study of the ketone (V) has shown that it is unquestionably a saturated compound; it gives negative reactions with tetranitromethane and the Liebermann–Burchard reagent, and is unaffected by prolonged treatment with perbenzoic acid. On the other hand, spectroscopic examination of it and of its parent thio-compound (for which the author is greatly indebted to Dr. R. A. Morton) reveals the presence of a strongly chromophoric grouping in these substances, which has apparently been produced by the removal of six atoms of hydrogen from β-amyrin. The compound (IV) shows maxima at 3210 A. ($\log \varepsilon = 4{\cdot}44$) and 2570 A. ($\log \varepsilon = 3{\cdot}88$), whereas the keto-acetate (V), in which the complicating effect of the sulphur atom is absent, shows a maximum at 2780 A. ($\log \varepsilon = 4{\cdot}08$). The absorption spectrum of (V) is thus similar in position to, although greater in intensity than, those of *neo*ergosterol (2680 A., $\log \varepsilon = 2{\cdot}5$; Inhoffen, *Annalen*, 1932, **497**, 130),

dihydrotrianhydrostrophanthidin (2680 and 2790 A., log ε = 2·7; Elderfield and Rothen, *J. Biol. Chem.*, 1934, **106**, 71), and dehydroabietic acid (2680 and 2760 A., log ε = 2·85; Fieser and Campbell, *J. Amer. Chem. Soc.*, 1938, **60**, 159). In the formation of these compounds aromatisation of an originally hydroaromatic ring has occurred; a similar aromatisation of β-amyrin to give (IV) and (V) provides the only adequate interpretation of the properties of the latter compound.

Although the benzoate of the ketone (V; BzO instead of AcO) is stated by Jacobs and Fleck to be stable towards permanganate in acetic acid, the ketone must be regarded as an intermediate stage in the conversion of the thio-compound into the $C_{30}$-lactone, because the latter has now been isolated (as acetate) in good yield as the sole product of the chromic anhydride oxidation of (V). (This oxidation product and the related hydroxy-lactone were identified by comparison with authentic samples kindly supplied by Dr. W. A. Jacobs.) The extreme smoothness of the oxidation, which can be effected under conditions of considerable severity, provides further and convincing evidence of the presence of an aromatic nucleus in the ketone (and consequently in the parent thio-compound), for only on this hypothesis can the stability under such drastic oxidative conditions of the partially dehydrogenated molecule be explained.

Of the four oxygen atoms in the $C_{30}$-lactone, three were characterised by Jacobs and Fleck as the lactone grouping and the original secondary hydroxyl group. The fourth oxygen atom, which these workers found to be unaffected by hydroxylamine and acylating agents, has now been shown to be ketonic, inasmuch as the acetate of the lactone has been converted by reduction with sodium and amyl alcohol into an unsaturated *acid*, $C_{30}H_{50}O_4$ (VII), m. p. 254°, which has been characterised by the preparation of its *methyl ester diacetate*, m. p. 219°. The formation of the latter compound ($C_{35}H_{56}O_6$) indicates the presence of two secondary hydroxyl groups and one carboxyl group in the acid $C_{30}H_{50}O_4$, and consequently the production of this acid from the lactone must involve the reduction of a carbonyl group in the latter, together with saponification of the lactone ring, elimination of the (tertiary) hydroxyl group thus liberated as water, and reduction of the aromatic nucleus. The mechanism of the formation of the $C_{30}$-lactone from the ketone $C_{30}H_{44}O_3$ is thus disclosed as a simple oxidation of the system >CH·CO— to >CO $HO_2C$—, with subsequent lactonisation on the tertiary hydroxyl group originally present. It therefore follows that the lactone must be formulated as $C_{30}H_{42}O_4$ instead of $C_{30}H_{44}O_4$ as given by Jacobs and Fleck, and that the partial structure (VI) may be assigned to it with a high degree of probability.* The acetate of (VI), as is to be anticipated from this structure, fails to give colour reactions indicative of unsaturation.




(VI.) (VII.)

The foregoing observations demonstrate the ability of β-amyrin to undergo aromatisation in one ring without loss of carbon atoms. The structure (I, R = H) is clearly irreconcilable with this behaviour, and accordingly requires modification by the removal of one or more methyl groups from quaternary positions therein. Aromatisation of ring E becomes possible if the methyl group at $C_{17}$ is shifted; in its new position, however, it must be non-quaternary, since all the available quaternary points are already occupied by four methyl groups, the ethenoid linkage ($C_{10}$—$C_{11}$ in β-amyrin; Ruzicka and Schellenberg, *Helv. Chim. Acta*, 1937, **20**, 1553), and the hydrogen atom at $C_6$ (required to interpret degradations in rings A and B). If the aromatic nucleus be derived from rings C or D, an extra methyl group can now be accommodated in ring E [as shown in (I, R = Me)],

* On the evidence at present available, the hydrogen atom eliminated in the production of (VII) from (VI) may be attached to the alternative carbon atom adjacent to the lactonised hydroxyl group.

but the removal of one or two methyl groups respectively to non-quaternary positions is still required. The only condition obviating removal of methyl groups to non-quaternary positions would be aromatisation of ring B, in which case the adoption of structure (I, R = Me) would account for one methyl group, and the remaining one could be attached to $C_{18}$. (This contingency is, however, regarded as remote, for reasons which will be considered in a future communication.)

The above argument is based on the assumption that no wandering of methyl groups has occurred during the reaction between β-amyrin and sulphur, which takes place under unusually mild conditions. This assumption must be regarded as not only justifiable in these circumstances, but necessary, for otherwise the diagnostic value of all dehydrogenation experiments would be seriously impaired.

An alternative possibility (instead of the removal of methyl groups to non-quaternary positions) would be the attachment of higher alkyl radicals to one or more quaternary points; the isolation of possible new dehydrogenation products should prove of value in deciding between these two alternatives.

## Experimental.

(Melting points are uncorrected.)

*Preparation of Thio-compound*, $C_{30}H_{44}OS$.—β-Amyrin benzoate, in batches of 10 g., was heated with an equal weight of sulphur in a stream of nitrogen as described by Jacobs and Fleck (*loc. cit.*), but the method of working up the product was somewhat modified, as follows: The reaction mixture was extracted 5 or 6 times with boiling chloroform, and the extract filtered and concentrated to small bulk (about 50 c.c. per batch). The cold solution was then filtered from deposited sulphur, concentrated further, and the crude thio-compound precipitated by addition of absolute alcohol (about 2 vols.). After one crystallisation from benzene–alcohol (1 : 2) the still impure material was refluxed for 1¾ hours with 6% benzene–alcoholic sodium hydroxide solution (20 vols.). After concentration to half-bulk the hydrolysed product was precipitated with water, filtered off, washed, and recrystallised from aqueous alcohol (charcoal). One further crystallisation (alcohol) yielded the practically pure thio-compound (usually in 55—60% yield) as soft, flat, colourless needles, m. p. 197·5—199°. Its solution in chloroform gave an intense brown colour with tetranitromethane, and with the Liebermann–Burchard reagent a deep brownish-red colour was produced.

*Preparation of Acetate*, $C_{32}H_{46}O_2S$.—The foregoing alcohol was dissolved in 1½ times its weight of pyridine and heated for 2 hours on the steam-bath with its own weight of acetic anhydride. After standing overnight, the solution was poured into aqueous methanol (1 : 1; 2 vols.) and the precipitated material recrystallised from 95% alcohol containing a little ether. This *acetate* is obtainable in two crystalline forms; it usually separates in long brittle prismatic needles, m. p. 197·5—198·5° after sintering and resolidifying at 185°, but occasionally forms rosettes of soft dull needles, m. p. 201—202°. A mixture of the two forms gives no depression in m. p., but each gives a marked depression when mixed with the parent alcohol (Found: C, 77·6; H, 9·3; S, 6·6. $C_{32}H_{46}O_2S$ requires C, 77·7; H, 9·4; S, 6·5%); $[\alpha]_D^{15°}$ + 98°, $[\alpha]_D^{12°}$ + 103° (m. p. 201—202°); $[\alpha]_D^{11°}$ + 111° (m. p. 197·5—198·5°) ($l$ = 1, $c$ = 2·72, 0·695, 4·00, in chloroform).

*Quantitative Saponification of Acetate*, $C_{32}H_{46}O_2S$.—The acetate (different preparations, and dried either at 85° in high vacuum or at 100° in an air-oven) was refluxed with a large excess (40 c.c.) of approximately 0·1N-alcoholic potash, and the alkali consumption determined by the method of Sandqvist and Gorton (*loc. cit.*). Found: $M$, 491·2 (mean of 5 values). Calc. for $C_{32}H_{46}O_2S$: $M$, 494·4. For cholesteryl acetate, $M$, 426·0 (mean of 3 values). Calc. for $C_{29}H_{48}O_2$: $M$, 428·4.

*Preparation of the Ketone*, $C_{30}H_{44}O_3$.—Potassium permanganate (22·5 g.) in water (400 c.c.) was added with mechanical stirring during 1½ hours to a solution of the acetate, $C_{32}H_{46}O_2S$ (10 g.), in glacial acetic acid (300 c.c.) at 20° approx.; towards the end of the addition the solution acquired a permanent pink colour. After a further 1½ hours the reaction mixture was clarified with sodium bisulphite and dilute sulphuric acid and poured into water (1100 c.c.). The solid product was filtered off, washed, and refluxed for 4 hours with a solution of sodium hydroxide (10 g.) in water (125 c.c.) and alcohol (250 c.c.). The solution was poured into water (1000 c.c.) and the crude neutral oxidation products were filtered off, washed, taken up in methyl alcohol, and decolorised with charcoal. The material obtained by concentration

and cooling was crystallised several times from aqueous methanol, but no evidence of the presence of the lactone, $C_{30}H_{42}O_4$ (isolated at this stage by Jacobs and Fleck) was obtained. Instead, the m. p. fell continuously from about 265° to roughly 220°. By combining and concentrating the filtrates from these crystallisations the ketone was obtained (in 25—30% yield) as long brittle needles, m. p. 282—283°. Jacobs and Fleck record m. p. 274—275° for this compound [a sample kindly supplied by Dr. Jacobs, however, melted at 277—278° (as stated by him in a private communication), and gave no depression on admixture with the preparation, m. p. 282—283°] (Found: C, 79·6; H, 9·6. Calc. for $C_{30}H_{44}O_3$: C, 79·6; H, 9·8%). Neither the ketone nor the crude mixture of neutral oxidation products gave a product on treatment with semicarbazide acetate under reflux.

*Keto-acetate*, $C_{32}H_{46}O_4$.—The ketone (1 part) was dissolved in pyridine (5 parts) and acetic anhydride (4 parts). The solution was maintained at 100° for 2 hours, water added, and the precipitate recrystallised from aqueous methyl alcohol. The acetate separated in heavy plates, m. p. 234—234·5° (Jacobs and Fleck give m. p. 231—232°) (Found: C, 77·8; H, 9·4. Calc. for $C_{32}H_{46}O_4$: C, 77·7; H, 9·4%). Its solution in chloroform remained colourless on addition of tetranitromethane; the Liebermann–Burchard reagent produced a clear, light brownish-yellow solution.

A solution of this acetate (50 mg.) in a large excess of an approximately 0·25N-solution of perbenzoic acid in chloroform was left at 5° for 14 days. The excess perbenzoic acid was then removed, and the product isolated in the customary manner; 40 mg., m. p. 230—232° (230—233° when mixed with the original acetate), were obtained after one crystallisation from aqueous methanol. This material was refluxed with 0·1N-alcoholic potash for 2 hours and yielded a product which separated from aqueous alcohol in needles, m. p. 285—286° both alone and on admixture with an authentic specimen of the ketone.

*Oxidation of the Keto-acetate*, $C_{32}H_{46}O_4$.—A solution of chromic anhydride (0·5 g.) in water (1 c.c.) and glacial acetic acid (14 c.c.) was added with stirring during 10—12 minutes to a solution of the keto-acetate (0·5 g.) in glacial acetic acid (10 c.c.) at 90°. After a further half-hour at this temperature the crystalline reaction product was precipitated by addition of water, washed, and recrystallised from slightly aqueous methanol, from which the pure lactone acetate separated in large plates, m. p. 284—286° (274—277° when mixed with Jacobs's lactone acetate of m. p. 269—271°); yield, 75%. The compound gave no colour with tetranitromethane in chloroform, and with the Liebermann–Burchard reagent a clear, very light red solution resulted (Found: C, 75·1; H, 9·1. Calc. for $C_{32}H_{44}O_5$: C, 75·5; H, 8·7%).

When heated under reflux for 2½ hours with 0·1N-alcoholic potash the above acetate was quantitatively converted into the free hydroxy-lactone, which separated from aqueous alcohol in small, heavy prismatic needles, m. p. 309—310° (decomp.) (305—308° on mixing with Jacobs's specimen of m. p. 302—303°) (Found: C, 76·6; H, 9·4. Calc. for $C_{30}H_{42}O_4$: C, 77·2; H, 9·1%).

*Preparation of the Acid*, $C_{30}H_{50}O_4$ (VII).—A solution of the acetate, $C_{32}H_{44}O_5$ (100 mg.), in hot amyl alcohol (5 c.c.) was treated with sodium (1 g.), added in portions during 50 minutes with addition of two 5 c.c. portions of amyl alcohol at intervals of 20 minutes. After a total of 1 hour the mixture was diluted with water and the amyl alcohol removed by distillation. The residual suspension (insoluble sodium salt) was extracted with ether, then acidified to Congo-red and re-extracted (ether). The residue from evaporation of the latter extract was thrice crystallised from aqueous methanol–acetone and yielded the *acid* as fluffy prismatic needles, m. p. 252—254° to a turbid liquid which on further heating partly resolidified and cleared finally at 280° (Found: C, 75·8; H, 10·6. $C_{30}H_{50}O_4$ requires C, 75·9; H, 10·6%).

Treatment of the acid with diazomethane yielded an amorphous ester which could not be induced to crystallise; this was accordingly heated with acetic anhydride in pyridine at 100° for 2 hours, and the crystalline product obtained by cautious dilution of the mixture was recrystallised from aqueous alcohol, from which the *methyl ester diacetate* separated in leaves, m. p. 217—219° (Found: C, 73·3; H, 9·8; OMe, 5·0. $C_{35}H_{56}O_6$ requires C, 73·4; H, 9·8; OMe, 5·4%). This ester gives an immediate yellow colour with tetranitromethane in chloroform, and with the Liebermann–Burchard test an intense, deep red coloration is immediately produced.

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