155. *The Triterpene Group. Part V. Oxidation Products of the β-Amyrin Derivative,* $C_{30}H_{44}OS$.

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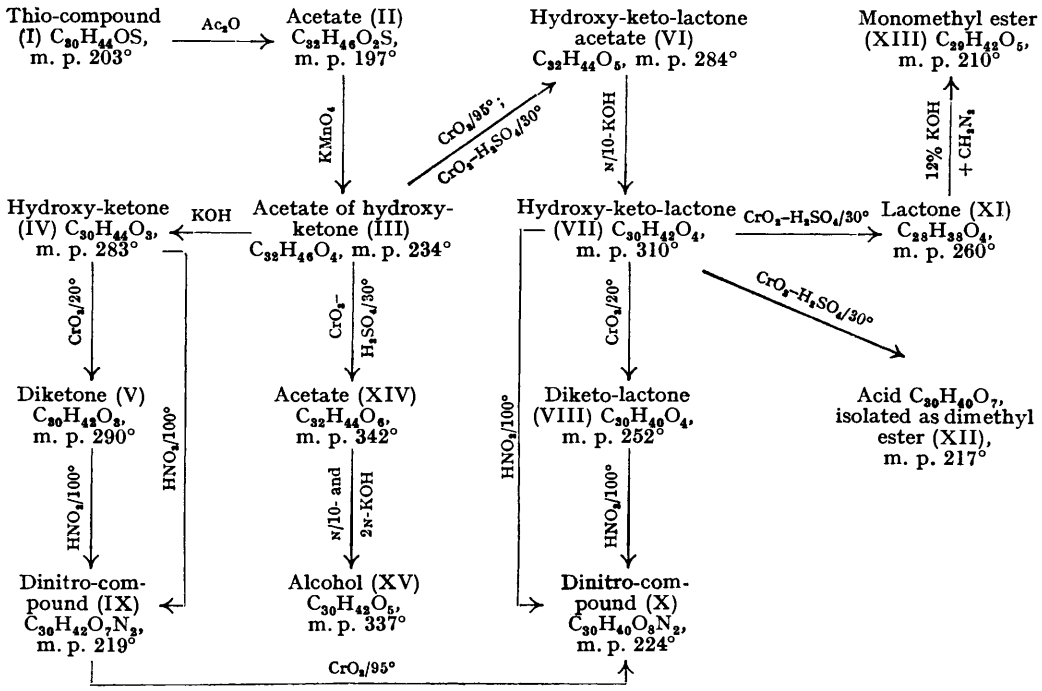
Improved conditions have been devised for the preparation, from β-amyrin benzoate, of the thio-compound $C_{30}H_{44}OS$ (I) and of the hydroxy-keto-acetate (III), the principal oxidation product of the acetate of (I). With a view to the elucidation of the structure of (I), the oxidation of (III) and its derivatives has been studied under various conditions. New products, at present incompletely characterised, have been obtained; the formation of some of these is difficult to explain by the current formulations of the oleanolic acid group of triterpenes.

In the hope of obtaining further insight into the structure of the compound $C_{30}H_{44}OS$ (I), first obtained by Jacobs and Fleck (*J. Biol. Chem.*, 1930, **88**, 137), the behaviour of its two immediate oxidation products, namely, the hydroxy-ketone (IV) and the hydroxy-keto-lactone (VII), on further oxidation has been studied.

The preparation of large amounts of the compound (I), which cannot be satisfactorily accomplished by the original method (fusion of β-amyrin benzoate with sulphur), has been achieved, with a greatly increased yield, by the use of benzyl acetate as a solvent. The preparation of the keto-acetate (III) also has been improved, the yield being raised to almost 50%.

Gentle oxidation of the hydroxy-ketone (IV) with chromic anhydride produces the *diketone* (V), characterised by its *monosemicarbazone*. This diketone is converted in good



yield into a *dinitro*-compound, $C_{30}H_{42}O_7N_2$ (IX), by the action of concentrated nitric acid at 100°; the latter compound is also formed, in the same yield, when the hydroxy-ketone (IV) is oxidised under the same conditions.

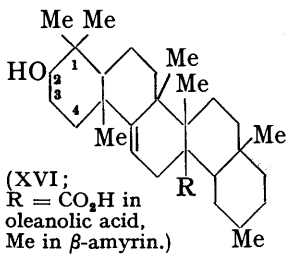
A similar series of changes has been carried out with the hydroxy-keto-lactone (VII). Oxidation of the C_2-hydroxyl group to carbonyl gives the *diketo-lactone* $C_{30}H_{40}O_4$ (VIII), which is transformed by nitric acid at 100° into a *dinitro*-compound, $C_{30}H_{40}O_8N_2$ (X), also obtained by similar oxidation of (VII).

The structural relationship between these two dinitro-compounds is apparently the same as that between the ketone (IV) and the lactone (VII) (Part II, J., 1938, 1313), because the compound (IX) on oxidation with chromic anhydride is smoothly converted into (X) under precisely the same conditions as those effective in transforming the keto-acetate (III) into the lactone-acetate (VI); this relationship is borne out by a comparison of the molecular formulæ of the two pairs of compounds. The nitro-compounds are remarkable in that the same compound is formed both from the alcohol [(IV) and (VII)] and from the ketone [(V) and (VIII)] by the action of nitric acid. The precise mechanism of the formation of these substances is obscure; the reaction may however be analogous to the oxidation of cholestenone recorded by Windaus (*Ber.*, 1906, **39**, 518) to a trinitro-derivative $C_{27}H_{41}O_6N_3$ by means of nitric acid–acetic acid at 100°, for he states that the same product may also be obtained from cholesterol.

A preliminary study has been made of the action of chromic anhydride on the acetate (III) in the presence of sulphuric acid. It was shown previously (Simpson, *loc. cit.*) that chromic anhydride (without sulphuric acid) converts (III) into (VI) in 75% yield. In marked contrast to this reaction, the addition of sulphuric acid lowers the yield of (VI) to about 15%, the main product being a new *acetate*, $C_{32}H_{44}O_6$ (XIV). Hydrolysis of this acetate with either dilute or concentrated potassium hydroxide solution gives the corresponding *alcohol*, $C_{30}H_{42}O_5$ (XV).

The presence of sulphuric acid also modifies profoundly the course of the oxidation of the lactone (VII). The diketo-lactone (VIII), referred to above, is the sole isolable product of the oxidation of (VII) by chromic anhydride without sulphuric acid at temperatures up to 40°. The addition of the mineral acid, however, brings about an enormous increase in the rate of oxidation, and leads to the formation, even at room temperature, of a complex mixture. The (very small) neutral fraction consisted essentially of the lactone acetate (VI). From the acid products of oxidation, a dibasic acid, presumably arising by scission of ring A at C_2—C_3, has been isolated in moderate yield as its *dimethyl* ester, $C_{32}H_{44}O_7$ (XII), together with a *monolactone* (XI), analyses of which indicate the formula $C_{28}H_{38}O_4$. This lactone appears to form water-soluble complexes with the alkali-metal salts of acids of allied type, as is shown both by its isolation from the acid oxidation products and also by its behaviour on alkaline hydrolysis. In the latter reaction it is converted into the corresponding acid, which was isolated as its *methyl* ester, $C_{29}H_{42}O_5$ (XIII). The lactone reacts readily with hydroxylamine, but no crystalline product has so far been obtained from this reaction. It does not react with semicarbazide acetate at room temperature.

The data here presented do not constitute direct evidence either for or against the mechanism previously suggested (Simpson, *loc. cit.*) for the production of (I), (III), and (VI). They do, however, accentuate the difficulty of explaining the formation of (I) and its transformation products on the basis of structure (XVI) (Ruzicka, Goldberg, and Hofmann, *Helv. Chim. Acta*, 1937, **20**, 325) for β-amyrin. In particular, structure (XVI) fails to account for the production of the monolactone (XI) with the loss of two carbon atoms. The discrepancy cannot, moreover, be bridged by any of the several variations of (XVI) which have recently been advanced (Haworth, *Ann. Reports*, 1937, **34**, 366; Beynon, Sharples, and Spring, J., 1938, 1233; Kitasato, *Acta Phytochim.*, 1938, **10**, 239; Ruzicka *et al.*, *Helv. Chim. Acta*, 1938, **21**, 1735; *Chem. and Ind.*, 1938, **57**, 1210; Picard, Sharples, and Spring, *ibid.*, 1939, **58**, 58). A detailed study is now being made of the optical and chemical properties of the compounds described in this paper, in the expectation that unequivocal evidence will be obtained bearing on the structure of (I)



(XVI; R = CO_2H in oleanolic acid, Me in β-amyrin.)

Experimental.

(Melting points are uncorrected; specific rotations were measured in chloroform.)

Improved Preparation of the Compound $C_{30}H_{44}OS$ (I).—A solution of β-amyrin benzoate (20 g.) and sulphur (10 g.) in benzyl acetate (60 c.c.) was refluxed (220—225°) under nitrogen for 4—4½ hours. The product was precipitated by addition of methyl alcohol–ethyl alcohol (2 vols., 1 : 1), recrystallised from benzene–alcohol, and hydrolysed as previously described (J., 1938, 1316). The yield of pure product (m. p. 202—203°) was 75% of the theoretical.

Improved Preparation of the Keto-acetate (III).—A solution of potassium permanganate (30 g.) in water (600 c.c.) was added during 2—2½ hours with stirring to a solution of the acetate (II) (20 g.) in glacial acetic acid (600 c.c.), care being taken to prevent the temperature (20°) from rising appreciably. After a further hour the solution was poured into water (2 l.), made acid to Congo-red with dilute sulphuric acid, and decolorised with sodium bisulphite. The suspension was then extracted with ether and the extract, after being washed with water and 2% sodium hydroxide solution, was dried and evaporated. The residue crystallised rapidly from methyl alcohol, and one recrystallisation yielded the practically pure keto-acetate, m. p. 233—234°, in 40—48% yield.

Diketone, $C_{30}H_{42}O_3$ (V).—A solution of the ketone (IV) (0·5 g.) in acetic acid (20 c.c.) and water (2 c.c.) was treated with chromic anhydride (0·21 g.) in acetic acid (14 c.c.) and water (1 c.c.), added at room temperature with stirring during 35 minutes. After 2 days the *diketone* was precipitated with water and recrystallised from aqueous alcohol or methanol, separating in long needles, m. p. 289—290° [272° when mixed with (IV)], $[\alpha]_D^{18°}$ − 94° (l = 1, c = 0·56) (Found: C, 80·1; H, 9·3. $C_{30}H_{42}O_3$ requires C, 79·9; H, 9·4%).

The *monosemicarbazone* crystallised from benzene–alcohol in small needles, m. p. 287—289° (decomp.) (272—282° when mixed with the diketone) (Found: C, 73·4; H, 9·1; N, 8·3. $C_{31}H_{45}O_3N_3$ requires C, 73·3; H, 8·95; N, 8·3%).

Diketo-lactone, $C_{30}H_{40}O_4$ (VIII).—A solution of chromic anhydride (1·5 g.) in water (4·5 c.c.) and acetic acid (25·5 c.c.) was added during ¾ hour with stirring to a solution of the lactone (VII) (4 g.) in acetic acid (90 c.c.) and water (10 c.c.) at room temperature. After 12 hours, water was added and the precipitated material was washed with hot water and crystallised from aqueous alcohol and then from benzene–light petroleum, from which the *diketo-lactone* separated in soft needles or hard prisms (according to the rate of crystallisation), m. p. 250·5—252°; from acetone it separated in long silky needles. $[\alpha]_D^{18°}$ + 66° (l = 1, c = 3·93) (Found: C, 77·0; H, 8·9. $C_{30}H_{40}O_4$ requires C, 77·5; H, 8·7%).

The same compound was obtained in approximately the same yield (60%) when the oxidation was carried out at 40°; at 70° the diketo-lactone underwent further oxidation.

The *monoxime* crystallised from chloroform–alcohol in small prismatic needles, m. p. 307—310° (decomp.) (Found: C, 74·5, 74·8; H, 9·1, 9·0; N, 2·9. $C_{30}H_{41}O_4N$ requires C, 75·1; H, 8·6; N, 2·9%).

Nitro-compound, $C_{30}H_{42}O_7N_2$ (IX).—(a) The keto-alcohol (IV) (1 g.) was warmed slightly with concentrated nitric acid (10 c.c.). After the initial reaction had subsided, the solution was heated in boiling water; a further rapid evolution of oxides of nitrogen occurred, and after 10 minutes a crystalline product separated. After being heated for a further 20 minutes the suspension was cooled. The *nitro*-compound was crystallised once from dilute acetic acid, and then from aqueous acetone, from which it separated in heavy irregular prisms, m. p. 219—220° (decomp.). Yield, 50%. $[\alpha]_D^{18°}$ − 87° (l = 1, c = 2·01) [Found: C, 66·7, 66·7, 66·9, 66·4; H, 7·5, 7·6, 7·5, 7·35; N, 5·1, 5·2 (several preparations). $C_{30}H_{42}O_7N_2$ requires C, 66·4; H, 7·8; N, 5·2%]. The compound gave negative tetranitromethane and Liebermann–Burchard reactions, and was recovered unchanged after treatment with diazomethane.

(b) The diketone (V) (250 mg.) was treated with concentrated nitric acid (2·5 c.c.). No oxidation occurred on slight warming, but at 100° a brisk reaction took place. The product crystallised after 10 minutes, and was purified, after a further 20 minutes' heating, as described above. It had m. p. 218—219° (decomp.) both alone and when mixed with the sample prepared by method (a). $[\alpha]_D^{18°}$ − 86° (l = 1, c = 2·35) (Found: C, 66·8, 66·8; H, 7·8, 7·6; N, 5·0%).

Nitro-compound, $C_{30}H_{40}O_8N_2$ (X).—(a) The lactone (VII) (300 mg.) was treated with concentrated nitric acid (3 c.c.), oxidation commencing after a few minutes at room temperature. The subsequent procedure was exactly as for the preparation of the nitro-compound (IX); the pure *compound* crystallised from aqueous acetone in sheaves of long needles, m. p. 223·5—224·5° (decomp.). $[\alpha]_D^{20°}$ + 49° (l = 1, c = 2·50) (Found: C, 65·3; H, 7·4; N, 4·5%).

(b) On addition of concentrated nitric acid (5 c.c.) to the diketo-lactone (VIII) (0·5 g.), no oxidation occurred at room temperature or on slight warming [as was also the case in the preparation of (IX) from the diketone (V)]. Oxidation was carried out under the conditions already specified, the product forming long needles from aqueous acetone, m. p. 223·5—224° (decomp.) either alone or mixed with the specimen prepared as in (a). $[\alpha]_D^{18°}$ + 45° (l = 1, c = 2·7) (Found: C, 65·15, 65·0; H, 7·7, 7·6; N, 4·7. $C_{30}H_{40}O_8N_2$ requires C, 64·7; H, 7·2; N, 5·0%). The compound was unaffected by diazomethane in ether–acetone solution.

(c) A solution of the compound (IX) (100 mg.) in acetic acid (5 c.c.) was oxidised at 95° with a solution of chromic anhydride (100 mg.) in water (0·3 c.c.) and acetic acid (4 c.c.), added in portions during ½ hour. After a further ½ hour water was added, and the precipitated material recrystallised from aqueous acetone. It separated in sheaves of long needles, m. p. 224—224·5° (decomp.) both alone and when mixed with the product prepared by method (b). $[\alpha]_D^{15°}$ + 50° (l = 1, c = 1·77).

Oxidation of the Lactone (VII) *with Chromic Anhydride–Sulphuric Acid.*—The lactone (4 g.) was dissolved in glacial acetic acid (100 c.c.) and concentrated sulphuric acid (3·5 c.c.), and a solution of chromic anhydride (4 g.) in water (6 c.c.) and acetic acid (60 c.c.) added with stirring during 50 minutes; the temperature rose from 20° to 30°. After 3 hours the solution was diluted with water and extracted with ether. The extract was washed with water and 2%

sodium hydroxide solution, dried, and evaporated. The residue (about 3% of the weight of lactone used) crystallised from aqueous methanol, and after several crystallisations from this solvent or from aqueous acetone the acetate (VI) was obtained in small irregular laminæ, m. p. 282—283° after slight previous shrinking. Its identity was confirmed by conversion into the free lactone (VII), m. p. and mixed m. p. 309—310° (decomp.).

The foregoing alkaline solution was re-extracted with ether (this extraction removed no ether-soluble material), and then acidified to Congo-red with hydrochloric acid and again extracted with ether. A solution in dilute acetic acid of the residue obtained by evaporation of the latter extract slowly deposited the new *lactone* (XI), which separated from methyl alcohol in brittle needles, m. p. 259—260°. The yield was somewhat variable, but in no case exceeded 10%. $[\alpha]_D^{14°} - 271°$ ($l = 1$, $c = 2{\cdot}21$) [Found (two preparations): C, 76·4, 76·4, 76·55; H, 8·8, 8·7, 8·6; *M* (Rast), 505, 501. $C_{28}H_{38}O_4$ requires C, 76·7; H, 8·7%; *M*, 438]. The lactone did not react with diazomethane in ether–acetone solution, and it gave negative tetranitro methane and Liebermann–Burchard reactions.

The original acetic acid filtrate from the lactone (XI) was diluted with water, and the precipitated acids isolated by means of ether. The mixture (the main bulk of the total oxidation products) showed no tendency to crystallise, but on treatment with diazomethane yielded a mixture of esters, which crystallised from aqueous methyl alcohol. Recrystallisation (aqueous methanol) of the mixture gave as the main fraction (25% of the weight of lactone oxidised) the *dimethyl* ester (XII), which formed large, brittle, elongated laminæ, m. p. 216·5—217·5°. $[\alpha]_D^{18°} - 31{\cdot}7°$ ($l = 1$, $c = 2{\cdot}86$) (Found: C, 70·9; H, 8·5; OMe, 11·3. $C_{32}H_{44}O_7$ requires C, 71·1; H, 8·2; OMe, 11·5%).

Hydrolysis of the Lactone (XI).—The lactone (180 mg.) was refluxed for 7 hours with alcoholic potassium hydroxide (12 c.c. of 12%). The solution was largely diluted with water and extracted with ether, but only a negligible quantity of ether-soluble material was obtained. The alkaline solution was acidified with acetic acid, and the product collected with ether. This material consisted of a mixture of unchanged lactone and the related acid, the former (approximately 40 mg.) being isolated by crystallisation from ether, in which it was sparingly soluble, and identified by m. p. and mixed m. p. The *methyl* ester (XIII) of the corresponding acid was prepared by treatment with diazomethane of the ethereal filtrate from the lactone; it crystallised in large soft plates from aqueous acetone, m. p. 210—211° (Found: C, 73·9, 74·0; H, 8·9, 8·9; OMe, 7·4, 6·9. $C_{29}H_{42}O_5$ requires C, 74·0; H, 9·0; OMe, 6·6%).

Oxidation of the Keto-acetate (III) *with Chromic Anhydride–Sulphuric Acid.*—Chromic anhydride (4 g.) in acetic acid (40 c.c.) and water (8 c.c.) was added in portions during ¼ hour to a solution of the acetate (III) (4 g.) in acetic acid (80 c.c.) and concentrated sulphuric acid (4 c.c.). After 12 hours the mixture was left for 2 days at 30° and then, after addition of methyl alcohol, diluted with water and extracted with chloroform–ether. After being washed with water, the extract was separated into acid and neutral fractions with 2% sodium hydroxide solution. The neutral solution was dried and evaporated, and the crystalline residue digested with methyl alcohol. The insoluble fraction, after two recrystallisations from benzene–alcohol, yielded the new *acetate* (XIV) in small soft leaflets, m. p. 342—344° (decomp.). $[\alpha]_D^{15°} + 63°$ ($l = 1$, $c = 1{\cdot}71$) (Found: C, 73·4, 73·5; H, 8·8, 8·9. $C_{32}H_{44}O_6$ requires C, 73·2; H, 8·5%). Yield, 25%. It was recovered unchanged after being refluxed for 4 hours with hydroxylamine acetate in benzene–alcohol.

The methyl-alcoholic filtrate from (XIV) was concentrated, and the product recrystallised from methyl alcohol, from which the lactone acetate (VI) separated in plates, m. p. 282—284°, not depressed by an authentic specimen.

Alcohol, $C_{30}H_{42}O_5$ (XV).—200 Mg. of the acetate (XIV) were heated under reflux with (*a*) 0·1N-alcoholic potassium hydroxide (50 c.c.) for 2 hours, and (*b*) 2N-alcoholic potassium hydroxide (12 c.c.) for 3½ hours. The product was isolated in each case by precipitation with water, and recrystallised from aqueous acetone–alcohol, from which the *alcohol* separated in small opaque cubes or prisms, m. p. 337—339° (decomp.) after previous shrinking; a mixture of this compound with (XIV) had m. p. 312—317°. $[\alpha]_D^{13°} + 26{\cdot}7°$ [sample from (*a*)], $+ 27{\cdot}4°$ [sample from (*b*)] ($l = 1$, $c = 0{\cdot}78$, 1·78 in pyridine). The alcohol was sparingly soluble in cold chloroform (Found: C, 74·6; H, 9·1. $C_{30}H_{42}O_5$ requires C, 74·6; H, 8·8%).

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