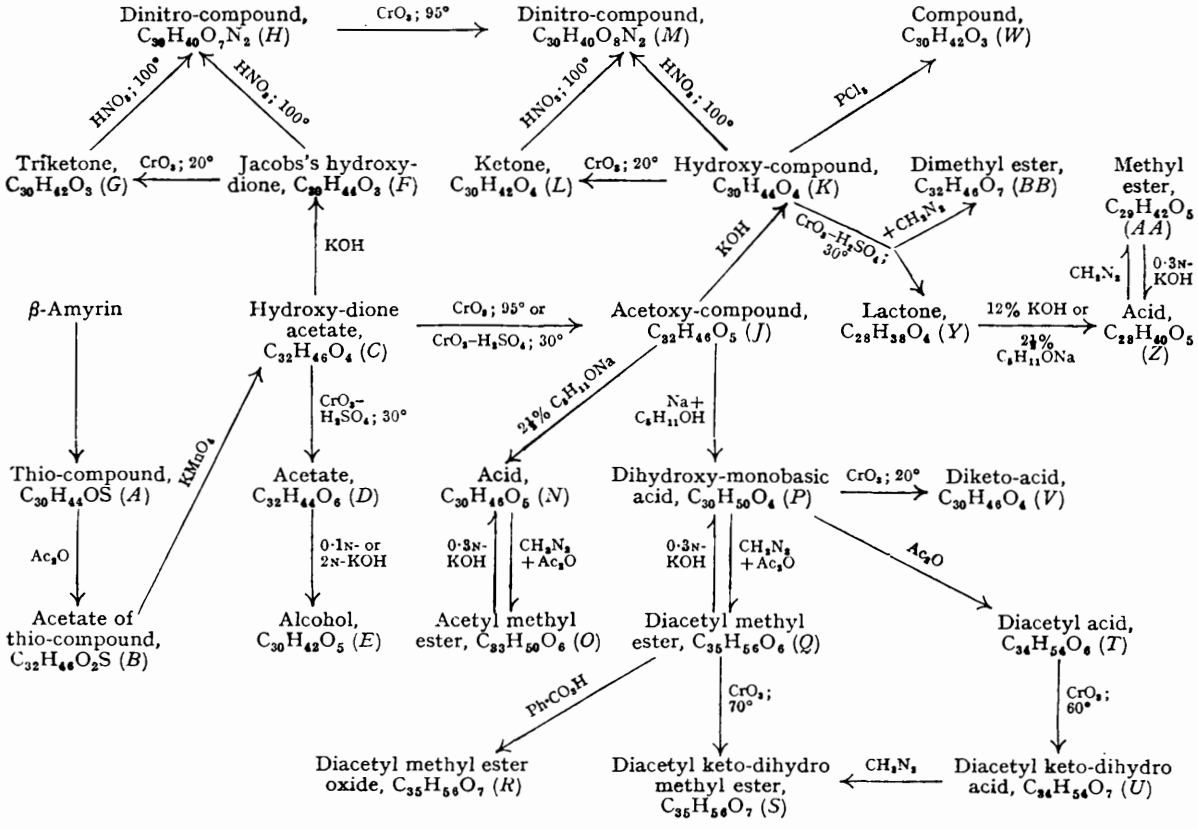
129. *The Triterpene Group. Part X. A Continuation of Parts II and V.*

By J. C. E. Simpson and R. A. Morton.

The studies initiated in Parts II and V have been extended (see chart below and Table II for summary). The previous hypothesis of the presence of an aromatic ring in the β-amyrin derivative (*F*) and its congeners is withdrawn, and the results obtained by a study of these compounds are critically considered with reference to the recent publications of Ruzicka, Kon, and their co-workers. It is concluded that neither the Ruzicka (I) nor the Kon (III) formulation of (*F*) accounts for the properties of certain derivatives of this substance, and this is regarded as due to incorrect siting of the chromophore of (*F*). The substances discussed have been examined both chemically and spectrographically and nitro-derivatives of the cholesterol series have been included in the survey for comparative purposes. Generalisations are made respecting the scope and limitation of the Liebermann–Burchard and the tetranitromethane reaction for the diagnosis of unsaturation in polycyclic hydroaromatic compounds.

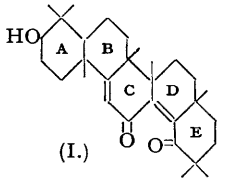
In Part II (J., 1938, 1313) one of us expressed the view that the compound $C_{30}H_{44}O_3$ (*F* in chart), obtained originally from β-amyrin by mild sulphur dehydrogenation and subsequent oxidation (Jacobs and Fleck, *J. Biol. Chem.*, 1930, **88**, 137), contains an aromatic ring. The evidence on which this conclusion was based consisted in the resistance to further oxidation of a supposed acetoxy-lactone (*J*) obtained from the acetate (*C*) of (*F*) by means of chromic anhydride, the absence of unsaturation reactions in both (*C*) and (*J*), and the position of the ultra-violet absorption maximum of (*C*). The intensity of this maximum (log ε = *ca.* 4) is clearly too high for an *isolated* aromatic ring, and it was considered that this factor might be due to the oxygen atoms in these substances being so located as to exert an auxochromic effect.* Continued study, however, showed that the dual properties of high-intensity absorption spectra and negative unsaturation tests persisted throughout an entire series of substances related to (*C*) and (*J*), and it became impossible to account for both sets of properties except by writing intrinsically improbable structures; in addition, efforts to isolate an aromatic



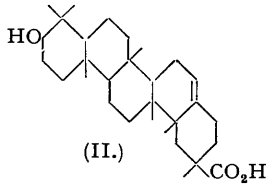
polybasic acid by nitric acid oxidation failed. At this point, the problem had to be temporarily laid aside. In the meantime, Ruzicka and Jeger (*Helv. Chim. Acta*, 1941, **24**, 1236) have shown that the compound (*F*)

* In Part II no attempt was made to give formal expression to this point owing to insufficient evidence. Commenting on this, Ruzicka, Müller, and Schellenberg (*Helv. Chim. Acta*, 1939, **22**, 767) have rightly pointed out the inadequacy of an isolated aromatic ring to account for the intensity of the absorption. On the other hand, Picard and Spring (J., 1941, 35, ll. 37, 38) state categorically that "an examination of its reactions led this author to the view that it [*i.e.*, (*F*)] contains an isolated benzenoid ring;" but such a view was not expressed in the paper to which they refer, and the phrase "isolated benzenoid ring" or its equivalent does not occur in it.

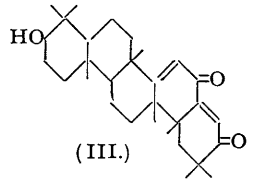
is not, as had been generally supposed, a keto-diol, but a hydroxy-dione, which condenses with hydrazine under drastic conditions to form a pyridazine derivative. It therefore contains two, and not three, double bonds, and the hypothesis of an aromatic ring is thus no longer tenable. On the basis of the spectrographic evidence and the pyridazine reaction, the Swiss authors have represented the hydroxy-dione (*F*) by the expression (I). Bilham, Kon, and Ross (J., 1942, 532, 535, 540) have adduced much evidence in favour of formula (II) for oleanolic acid, on the basis of which, as Kon and Ross (J., 1942, 741) have pointed out, the hydroxy-dione must be represented as (III). In view of these recent publications we record our own uncompleted experiments, which were carried out mainly in 1939 and 1940.



(I.)



(II.)



(III.)

The compound (*J*) was originally obtained in rather impure condition by Jacobs and Fleck (*loc. cit.*) as a concomitant of the hydroxy-dione by permanganate oxidation of the parent thio-compound (*A*, as benzoate). As Jacobs's hydroxy-dione was believed to be a keto-diol, the formation of (*J*) was explained (J., 1938, 1313) by the mechanism $>CH\cdot CO^- \longrightarrow >CO\ HO_2C^-$, with subsequent lactonisation on the (tertiary) hydroxyl group, and it was accordingly formulated as $C_{32}H_{44}O_5$, derived from $C_{32}H_{46}O_4$. However, all of many analyses which have been obtained for the acetoxy-compound (*J*), the hydroxy-compound (*K*), the related ketone (*L*) (carbonyl in position 2), and the oxime of the last, consistently favour a formula with two additional hydrogen atoms, as can be seen from Table I. A formula $C_{30}H_{44}O_4$ is in fact demanded by any expression for an O_4-

TABLE I.

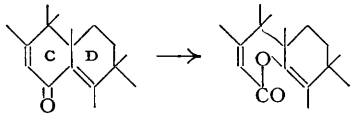
Acetate (*J*).		Alcohol (*K*).		Ketone (*L*).		Oxime.	
C, %.	H, %.	C, %.	H, %.	C, %.	H, %.	C, %.	H, %.
75·06, 75·02, 75·11, 74·87, 75·06, 75·11,* 75·23,* 75·17 *	8·84, 8·85, 9·09, 9·16, 8·94, 8·98, 9·12, 8·86	76·61,† 76·25 †	9·39, 9·22	76·95, 76·90, 76·97	8·92, 8·92, 9·06	74·49, 74·77	9·12, 9·02
Calc. for $C_{32}H_{46}O_5$: 75·26	9·08	Calc. for $C_{30}H_{44}O_4$: 76·87	9·47	Calc. for $C_{30}H_{42}O_4$: 77·20	9·08	Calc. for $C_{30}H_{43}O_4N$: 74·80	9·00
Calc. for $C_{32}H_{44}O_5$: 75·55	8·72	Calc. for $C_{30}H_{42}O_4$: 77·20	9·08	Calc. for $C_{30}H_{40}O_4$: 77·53	8·68	Calc. for $C_{30}H_{41}O_4N$: 75·11	8·58

* Ruzicka, Müller, and Schellenberg, *loc. cit.*

† These are the two highest (for carbon) of a series of analyses by Weiler and by Schoeller of various samples prepared from the pure acetate.

lactone which can be written on the basis of (I) or (III), and it thus appeared that the oxidation of (*C*) to (*J*) consisted in the process

$>CO\cdot\overset{|}{C}=\overset{|}{C}- \longrightarrow >CO\cdot O\cdot\overset{|}{C}=\overset{|}{C}-$, *e.g.*,

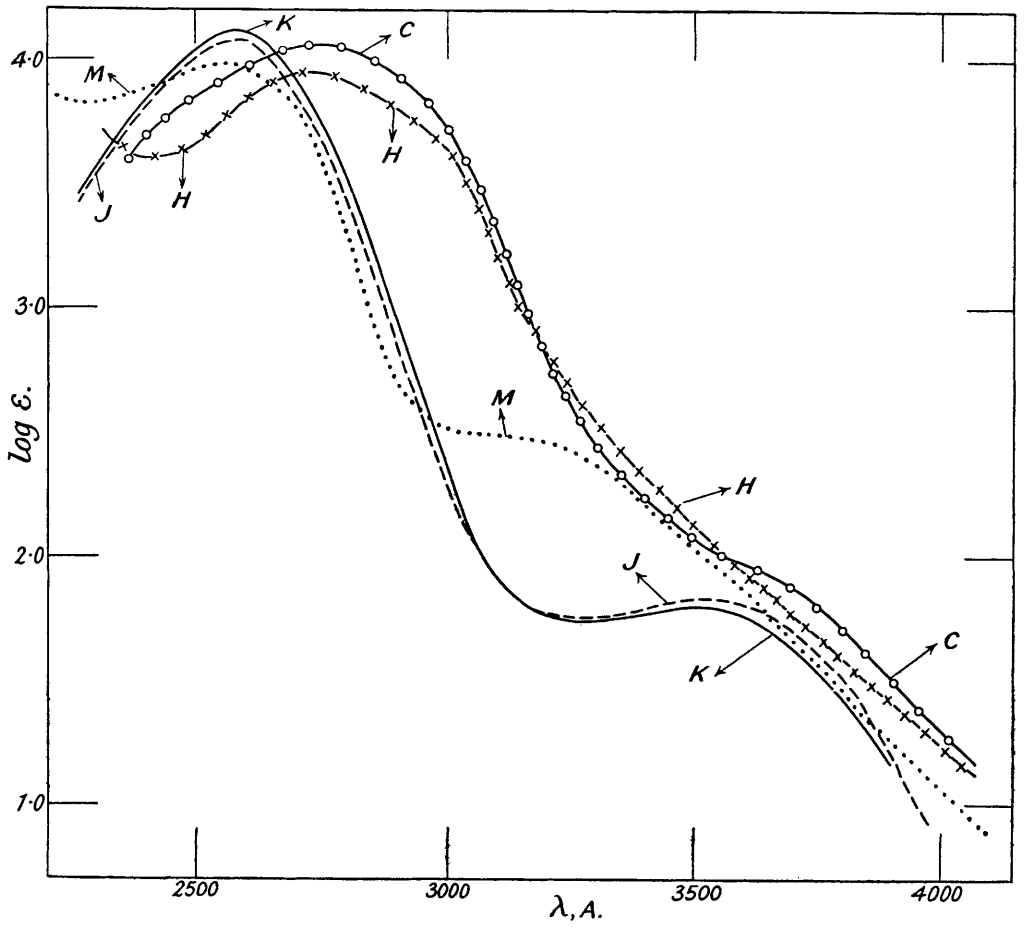


However, in a paper (*Helv. Chim. Acta*, 1942, **25**, 1409) which has just come to hand, Ruzicka and Jeger have shown that (*J*) yields the same pyridazine derivative (VI) as (*C*), from which it follows that (*J*) contains the original cyclic skeleton of (*C*), and consequently cannot be a lactone. It must therefore be represented as the acetate of an oxide, $C_{30}H_{44}O_4$ (IV) or (V), the latter being favoured by the Swiss authors.

Kon's formulation (III) of (*C*) would similarly give rise to (VII) or (VIII) for (*J*), and to (IX) for the pyridazine derivative. The absorption spectrum of (*J*) is in better agreement (see Fig. 1, also Ruzicka, Müller, and Schellenberg, *loc. cit.*) with (V) and (VIII) than with either (IV) or (VII). A much more decisive discrimination between these alternatives, however, is provided by the absorption spectrum (Fig. 2) of the acid (*N*), $C_{30}H_{46}O_5$, to which the keto-oxide (*K*) gives rise on energetic hydrolysis. Jacobs and Fleck (*loc. cit.*) found that (*K*) was unaffected when boiled with 2N-alcoholic potassium hydroxide or when heated with 5% alcoholic alkali at 130°, but was hydrolysed if the concentration of the alkali in the latter case was 10%. Ruzicka and Jeger (*loc. cit.*, 1942) have also prepared the acid, but found that more drastic conditions (10% methyl-alcoholic alkali at 200—210°) were required than those given by the American workers. We ourselves obtained the acid some three years ago by the convenient method of gentle refluxing with 2½% sodium amyloxide.

The properties of this acid, which was not studied by Jacobs and Fleck, are very characteristic; in contrast to the parent keto-oxide, it is very sensitive towards oxidising agents, and is slowly affected even by the atmosphere. It has a pronounced yellow colour, gives an intense ferric reaction in alcohol and a yellow colour with tetranitromethane [(*J*) does not give either of these colour reactions], and shows strong and unusual absorption in the ultra-violet (Fig. 2). All these properties are closely simulated by the related *acetyl methyl* ester (*O*),

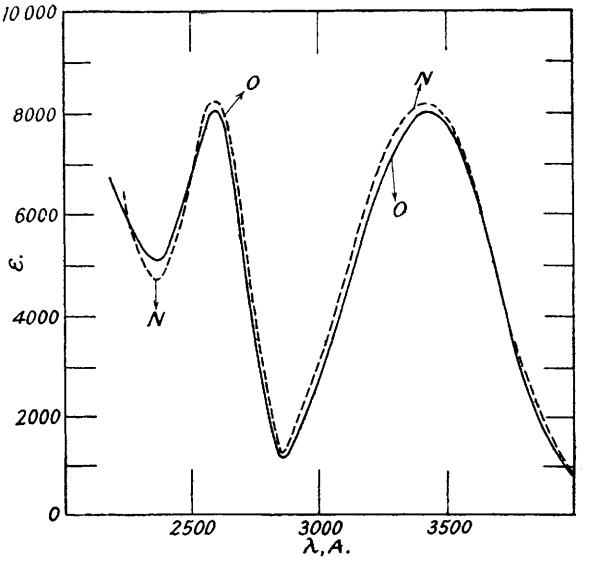
FIG. 1.



which yields unworkable resins on treatment with acetic acid solutions of chromic anhydride (instantaneous oxidation at room temperature), and warm hydrogen peroxide and lead tetra-acetate; the ester contains one active hydrogen atom (Zerewitinoff determination).

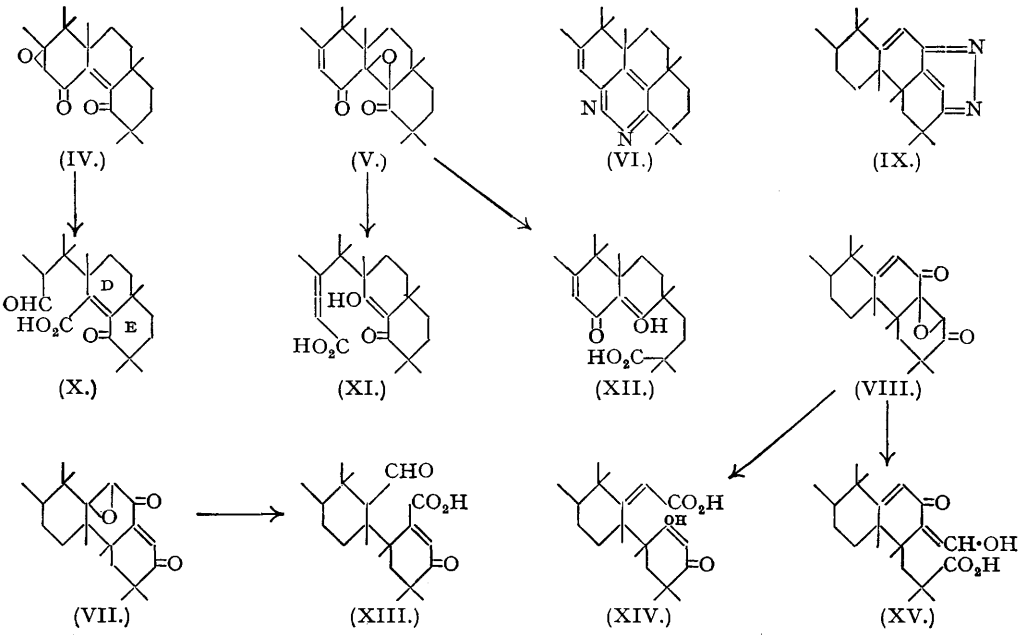
Ruzicka and Jeger also recorded the above colour and colour reactions for this acid and state that it contains three active hydrogen atoms; further, they attributed a change in absorption spectrum on keeping to an enol ⟶ keto conversion. This may well be a contributory factor in the remarkable change, but we believe that atmospheric oxidation is also involved, as old samples of the acid and its acetyl methyl ester (*O*) both gave significantly low carbon values on combustion, and their m. p.'s had also fallen appreciably.

FIG. 2.



Now the three possible structures for the acid (*N*) which can be derived from (IV) and (V) are (X), (XI), and (XII); but since (X) does not account for the ferric reaction shown by (*N*) and (*O*), it must be rejected. Structures (XI) and (XII), on the other hand, are both enolised β-diketones, thus accounting for the ferric reaction, but the conjugation depicted in (XI) is inadequate to account for the absorption spectra of (*N*) and (*O*). The chromophore of (XII), which is the formula advocated by Ruzicka and Jeger, is similar to that of ethyl α-mesityloxidoxalate, $Me_2C{:}CH{\cdot}CO{\cdot}CH{:}C(OH){\cdot}CO_2Et$ (Morton, Hassan, and Calloway, J., 1934, 898), which, in presence of excess sodium ethoxide, shows maxima at 2495, 2870, and 3510 A. (ε = 7500, 7500, and 13,000, respectively).

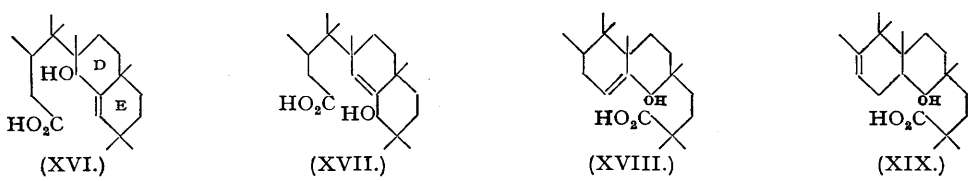
Thus there is at least good qualitative agreement at *ca.* 2500—2600 and at 3500 A. between this compound and the substances (*N*) and (*O*). In the absence of sodium ethoxide, however, the absorption spectrum of



ethyl α-mesityloxidoxalate is markedly different ($\lambda_{max.}$ at 3120 A., $\varepsilon = 14{,}000$); this may of course be due to an enol-keto change, but, if this is so, then Ruzicka and Jeger's suggestion that (*N*) changes into the keto-form on keeping is probably incorrect, as the maxima given by these authors for an old specimen of (*N*) are at 2350 and 3400 A. (log $\varepsilon = 4{\cdot}05$ and $3{\cdot}0$, respectively). These optical discrepancies cannot perhaps be considered seriously to militate against (XII) as a representation of (*N*), but this structure fails to account for a number of other observations discussed below.

First, we have found that the acetyl methyl ester (*O*) can be readily reconverted into the acid (*N*) by means of 0·3N-alcoholic alkali under reflux. Although the activating effect of carbonyl groups and ethylenic linkages on the alkaline hydrolysis of esters is often not predictable, it is extremely unlikely that the substitution shown in the γ-position of an αα-dimethylbutyric ester (XII) could facilitate hydrolysis to such an extent as actually occurs with the ester (*O*)

Secondly, an argument against the structure (XII) for the acid (*N*) is provided by the properties of the unsaturated dihydroxy-monobasic acid, $C_{30}H_{50}O_4$ (*P*), previously obtained by one of us (J., 1938, 1313) by reduction of the acetoxy-compound (*J*) with sodium and amyl alcohol. Various expressions, which need not be detailed, can be derived for this acid (*P*) on the basis of (IV) and (V), but in each of them the carboxyl group will in all probability be identical with that of the acid (*N*), and this carboxyl evidently cannot be tertiary, because the acid (*P*) is readily produced from its diacetyl methyl ester (*Q*) by boiling it with 0·3N-alcoholic alkali. In contrast to this facile hydrolysis, Bryant and Smith (*J. Amer. Chem. Soc.*, 1936, **58**, 1014), in a systematic study of the saponification of numerous esters, found that ethyl pivalate, the simplest case of an ethyl ester of a tertiary acid, requires 2 hours' heating at 100° under pressure with 2N-methanolic alkali for complete hydrolysis. Furthermore, the ease of hydrolysis of (*Q*) cannot be ascribed to any activating influence on the part of the remaining functional groups in the molecule, for this substance is optically transparent, and oxidation of the acid (*P*) with chromic anhydride yields a *diketo-acid*, $C_{30}H_{46}O_4$ (*V*), which shows only the weak selective absorption in the ultra-violet characteristic of unconjugated carbonyl groups (Fig. 3). The absence of conjugation in this diketo-acid is important, because it effectively counters the only possible objection to the foregoing argument against formula (XII), *viz.*, that ring-fission of (*J*) may occur in two different directions according to whether this substance is hydrolysed with sodium amyloxide to give (*N*), or reduced



with sodium and amyl alcohol to yield (*P*). For if (XII) were correct, and (*P*) were assumed to arise by a different ring-scission (of ring C), the only two possible structures for (*P*) would be (XVI) and (XVII). In

each of these, the position of the double bond with respect to the hydroxyl group is fixed by the adjacent angle methyl groups, and αβ-unsaturated ketones would therefore result on oxidation, whereas, as already mentioned, an unconjugated product is actually obtained.

Turning now to Kon's formula, (*J*) will be represented by (VII) or (VIII), and (*N*) by (XIII), (XIV), or (XV), and each of the last three is open to the same objections as (X), (XI), and (XII), respectively; ***i.e.***, (XIII) would not give a ferric reaction, (XIV) is inadmissible on optical grounds, and (XV) fails to account for the facile hydrolysis of (*O*) and (*Q*) and for the absence of conjugation in the diketo-acid (*V*).

The diacetyl methyl ester (*Q*) gives an *oxide*, $C_{35}H_{56}O_7$ (*R*), on treatment with perbenzoic acid; oxidation with chromic anhydride, on the other hand, yields an *isomer* (*S*), which shows the weak absorption (Fig. 3) indicative of an isolated carbonyl group. The same product (*S*) is obtainable from the acid (*P*) by the alternative route of acetylation, oxidation, and methylation; the oxidation product (*U*) of the diacetyl acid (*T*) showed an absorption spectrum (Fig. 3) indicating the presence of about 20% of an αβ-unsaturated ketone as contaminant, which was removed by methylation and recrystallisation of the mixture. The double bond in the

FIG. 3.

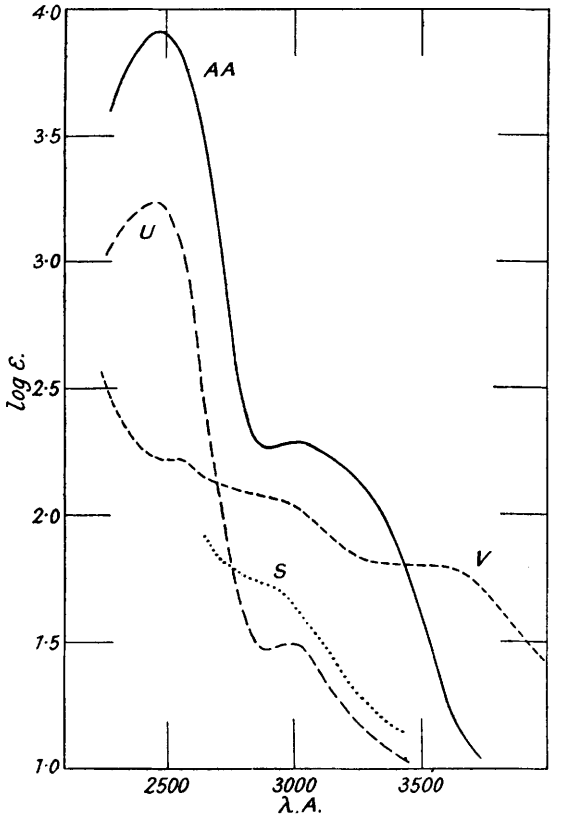
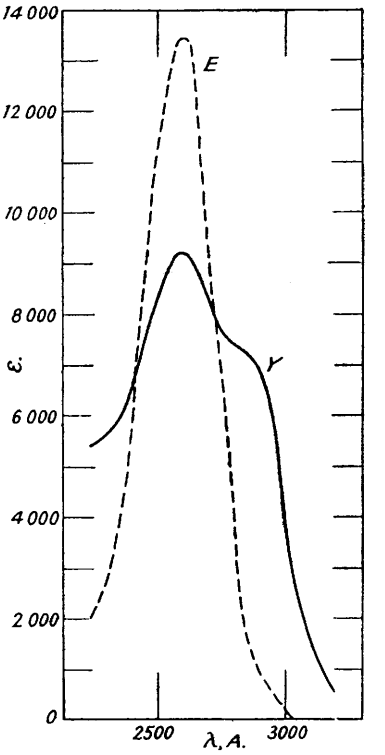


FIG. 4.



acid (*P*) thus appears to undergo two simultaneous types of oxidation, *viz.*, $>C{:}CH{\cdot}CH_2^- \longrightarrow >CH{\cdot}CO{\cdot}CH_2^-$ (mainly) and $>C{:}CH{\cdot}CO^-$, as so often occurs with the natural triterpene acids and alcohols. The position of the main band (2450 A.) of the absorption curve of (*U*) is fully in harmony with the view that the double bond of (*P*) is susceptible to this bi-directional oxidation, for it indicates that the αβ-unsaturated ketone, formed as the minor product, is of the disubstituted type $\overset{a}{b}\!>\!C{=}CH{\cdot}CO^-$ (*Ann. Reports*, 1941, **38**, 18). It may be noted that, of all the formulations of (*P*) derivable from (V) and (VIII), only two, (XVIII) and (XIX), fulfil this condition, and these are excluded for reasons already given.

The foregoing considerations lead to the conclusion that neither (I) nor (III) is a correct representation of the hydroxy-dione (*F*). We regard the inadequacy of these structures to explain our observations as due, not to the actual nature of the chromophore—the experiments of Ruzicka and Jeger (*loc. cit.*, 1941) would seem to be quite conclusive on this point—but rather to the fact that the chromophore is incorrectly sited either in the molecule as a whole or with reference to neighbouring angle methyl groups.

A further difficulty, which gives additional substance to these conclusions, is experienced when one attempts to formulate, on the basis of (I) or (III), the lactone (*Y*), to which the formula $C_{28}H_{38}O_4$ was ascribed in Part V (J., 1939, 755). This lactone is obtained in small amount when the substance (*K*), $C_{30}H_{44}O_4$, is oxidised with chromic anhydride in presence of sulphuric acid. Six analyses by two analysts (A. Schoeller and W. F. Boston)

have now been carried out on three different samples of the lactone, and have given, as extreme values, C, 76·52, 76·10 (mean 76·34); H, 8·79, 8·49 (mean 8·64%), the means agreeing more closely with the values (76·66 and 8·74%) required by $C_{28}H_{38}O_4$ than with any other formula; this formula is also supported by analytical data obtained for the monobasic *acid* (*Z*) and the methyl ester (*AA*) previously described (*loc. cit.*). Unfortunately, this lactone is not available in quantity, many experiments having failed to improve the yield, which in no case exceeds 10% of the precursor (*K*); hence, it has not yet been possible to characterise the two remaining oxygen atoms, but the following points have been established.

Hydrolysis of (*Y*) with 12% alcoholic potassium hydroxide and also with 2½% sodium amyloxide gives the same product (*Z*). This hydrolysis, in sharp contrast to that of (*J*), produces no radical change of chromophore, as may be seen by comparing the absorption spectrum of (*Y*) with that of the related ester (*AA*) (Figs. 4 and 3 respectively); correspondingly, neither this ester nor the related acid (*Z*) gives a coloration with ferric chloride, in contrast to substances (*O*) and (*N*). The esters (*AA*) and (*O*), however, resemble each other in the ease with which they undergo alkaline hydrolysis to the parent acids. The ester (*AA*) does not react with acetic anhydride in pyridine at 100° or with semicarbazide acetate in boiling alcohol; the lactone (*Y*) is likewise unaffected by semicarbazide and phenylhydrazine acetates. It is not certain that the carboxyl liberated in the hydrolysis of (*Y*) is identical with that present in the acids (*N*) and (*P*), but it is very probable that, in the formation of (*Y*), ring A has been oxidised beyond the ketone stage (we do not know of any instance of failure of the C_2-carbonyl to react with ketonic reagents). The implications arising from these observations are important, but cannot profitably be pursued until the functional groups of (*Y*), (*Z*), and (*AA*) have been fully diagnosed.

The dimethyl ester (*BB*), obtained from the acid fraction accompanying the formation of (*Y*), is formed by the rupture of ring A of (*K*) at C_2–C_3. Since, as has been shown above, the formula of the latter is now established as $C_{30}H_{44}O_4$, it follows that the formula of (*BB*), originally given (*loc. cit.*) as $C_{32}H_{44}O_7$, must be revised to $C_{32}H_{46}O_7$ (Found: C, 70·9; H, 8·5. $C_{32}H_{46}O_7$ requires C, 70·8; H, 8·6%).

We have already (p. 477) noted the persistence of high-intensity absorption spectra together with the absence of unsaturation reactions (Liebermann–Burchard and tetranitromethane tests) throughout many of the substances under discussion. This behaviour is summarised in Table II. On the whole, there are no inconsistencies

TABLE II.

Substance (see chart).	Liebermann–Burchard reaction.	$C(NO_2)_4$ reaction.	Absorption spectra. λ, max., A.	Absorption spectra. log ε, max.
C	+ weak	−	2740 ~3600	4·05 1·95
D	+	−		
E	+	−	2600	4·13
F	+	−		
G	−	−		
H	−	−	2735	3·94
J	+ weak	−	2585 3480	4·07 1·83
K	+	−	2585 3465	4·11 1·80
L	−	−		
M	−	−	2550 3160	3·99 2·47
N	+ weak	+	2600 3430	3·92 3·92
O	+ weak	+	2590 3465	3·91 3·91
P	+	doubtful		
Q	+	+		
R	+	−		
S	+	−	~2850	1·75
T	+	+		
U			2450 2970	3·24 1·50
			2390 2530 3550	2·27 2·22 1·85
V	+	+		
W	+	+		
Y	−	−	2600 2820	3·96 3·87
Z	−	−		
AA	−	−	2480 3000	3·90 2·295
BB	+ weak	−		

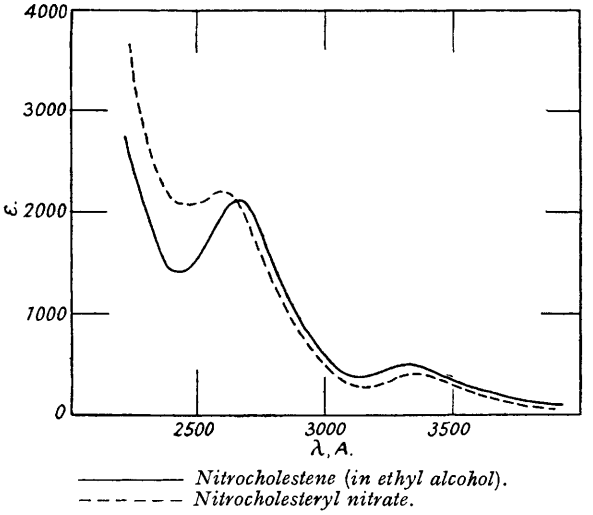
between the actual responses to the tetranitromethane test and those that might be expected on the basis of the types of chromophore present, in so far as these are known. Thus, the acid (*P*) is the parent compound of a group of substances of which the majority give positive tetranitromethane reactions. It has already been shown that (*P*) contains an isolated double bond, and, apart from the abnormally weak response of the acid itself, there is nothing unusual in the behaviour of its congeners in the tetranitromethane test. With the exception of this group of compounds, there are only three instances of positive tetranitromethane reactions in Table II, *viz.*, (*N*), (*O*), and (*W*). Of these, (*W*), from its mode of formation, obviously contains an isolated double bond which has been introduced by the well-known dehydrating action of phosphorus pentachloride on triterpene derivatives hydroxylated at C_2 (compare Vesterberg, *Ber.*, 1887, **20**, 1247; Nöjd, *Arch. Pharm.*, 1927, **265**, 381; Winterstein and Stein, *Annalen*, 1933, **502**, 223), while in (*N*) and (*O*) the chromophore of (*J*) has been radically changed, as has been shown above. The nature of the chromophore in the series (*Y*), (*Z*), and (*AA*) cannot yet be deduced with certainty, so no comment on their negative tetranitromethane reactions in relation to structure can be made, apart from the obvious inference that isolated ethylenic linkages are absent.

Examination of the data in Table II on the Liebermann–Burchard reaction, in conjunction with the chart, shows that these data can be rationally interpreted on the basis of Ruzicka and Jeger's chromophore for (*C*)

by making two postulations, *viz.*, (i) that, in polycyclic hydroaromatic compounds, conjugated systems other than those composed of a pair of ethylenic linkages give negative Liebermann–Burchard reactions; and (ii) that triterpene derivatives containing a hydroxyl or an acetoxyl group attached to C_2 give positive Liebermann–Burchard reactions irrespective of the structure of the remainder of the molecule.

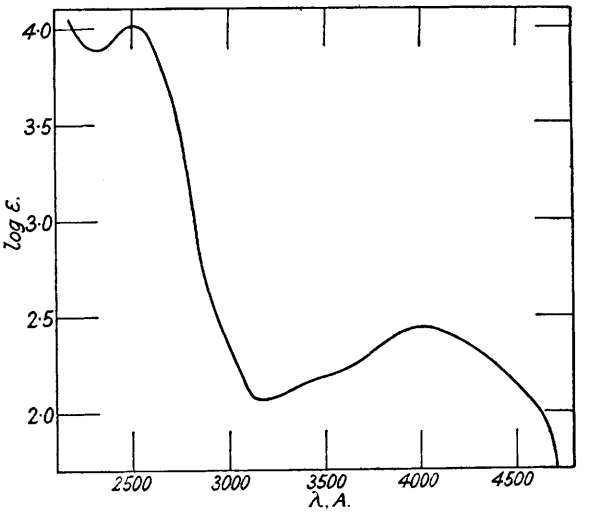
Ruzicka, Goldberg, and Wirz (*Helv. Chim. Acta*, **1935**, **18**, 61) state that the saturated triterpene alcohols dihydrobetulin and dihydrolupeol give positive responses in the Liebermann–Burchard test, but the validity of this observation *per se* as evidence for (ii) is discounted by the well-known difficulty of removing the final traces of unsaturated precursors during catalytic reductions. However, a comparison of the acetoxy- and hydroxy-diones (*C*) and (*F*) with the triketone (*G*), and of the acetoxy- and hydroxy-compounds (*J*) and (*K*) with the corresponding ketone (*L*), leaves no room for doubt that acetoxyl and hydroxyl groups attached to C_2 give rise to centres of unsaturation in this reaction. Similarly, each member of the series (*Y*), (*Z*), and (*AA*), in which the hydroxyl in ring A has been removed by oxidation, gives a negative reaction. The ready removal of the elements of water (or acetic acid) from the hydroxylated (or acetoxylated) ring A in the Liebermann–Burchard reaction is obviously comparable with the facile dehydration by means of phosphorus pentachloride already referred to. This instability in acid media is peculiar to the triterpene type of molecule among polycyclic hydroaromatic compounds; in the sterol group, on the other hand, it is well established that saturated members give negative Liebermann–Burchard reactions, and that they react normally with phosphorus pentachloride to yield the saturated chlorides.

FIG. 5.



——— *Nitrocholestene (in ethyl alcohol).*
– – – – – *Nitrocholesteryl nitrate.*

FIG. 6.

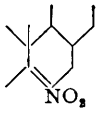
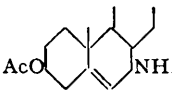


Windaus's compound, $C_{27}H_{41}O_6N_3$, *in alcohol.*

Having regard to the negative Liebermann–Burchard reactions shown by (*G*), (*L*), (*Y*), (*Z*), and (*AA*), to what has already been said respecting the chromophores in these substances, and to the absorption spectra exhibited by these chromophores, the necessity for postulate (i) is self-evident. The generalisation expressed therein has not previously been made either in the sterol or in the triterpene series; indeed, before we were convinced of its truth, we regarded the negative reactions of the substances named as being explicable only on the assumption that these compounds contained an aromatic ring. It will be noted that the nitro-compounds (*H*) and (*M*) also give negative Liebermann–Burchard reactions, and in this connexion we were led to examine nitro-derivatives of the cholesterol series. Although compounds such as nitrocholestene and nitrocholesteryl nitrate have always been represented as unsaturated (XX), the evidence for this does not appear to be entirely conclusive, as it rests largely on the analogy between their reduction to 6-cholestanones and that of nitroindene to β-hydrindone (cf. *Ann. Reports*, 1927, **24**, 131). We have examined the behaviour of nitrocholestene, nitrocholesteryl nitrate, and the compound $C_{27}H_{41}O_6N_3$ [prepared by Windaus (*Ber.*, 1906, **39**, 518) by the action of hot nitric acid on either cholesterol or cholestenone] in the tetranitromethane and the Liebermann–Burchard reaction, and find that all three substances show negative responses; furthermore, nitrocholestene fails to react with perbenzoic acid under the usual conditions. On the other hand, the presence of an isolated (*i.e.*, unconjugated) nitro-group in nitrocholestene and in nitrocholesteryl nitrate is effectively ruled out by their absorption spectra (Fig. 5), which demonstrate conclusively that these substances contain conjugated systems, as represented by (XX); their negative unsaturation reactions are therefore legitimate evidence in favour of the generalisation (i) given above. The constitution of Windaus's compound $C_{27}H_{41}O_6N_3$ is not known; * spectrographically (Fig. 6) it resembles rather strikingly the nitro-compound (*H*) (Fig. 1), but this

* A further point of resemblance between these substances is that each of them—as we have confirmed in the case of $C_{27}H_{41}O_6N_3$—arises by treatment of either the alcohol or the ketone with nitric acid, suggesting the presence of the grouping $O_2NO\cdot\overset{|}{C}=\overset{|}{C}\cdot NO_2$.

may be fortuitous, as the major chromophore in (*H*) appears to be the nitrogen-free system characteristic of (*C*) and (*F*). From analogy with nitrocholestene and nitrocholesteryl nitrate, substances (*H*) and (*M*) are

(XX.)  (XXI.)   (XXII.)

formulated as unsaturated derivatives (XXI), but neither the spectrographic (Fig. 1) nor the analytical data are decisive on this point, although the latter are in better agreement with the H_{40} (unsaturated) than with the H_{42} formulæ [For (*H*): $C_{30}H_{40}O_7N_2$ requires C, 66·64; H, 7·46. $C_{30}H_{42}O_7N_2$ requires C, 66·39; H, 7·80. Found (mean of six analyses): C, 66·72; H, 7·56. For (*M*): $C_{30}H_{40}O_8N_2$ requires C, 64·71; H, 7·24. $C_{30}H_{42}O_8N_2$ requires C, 64·49; H, 7·58. Found (mean of three analyses): C, 65·15; H, 7·57%].

Finally, Eckhardt (*Ber.*, 1938, **71**, 461) states that 7-acetamidocholesteryl acetate (XXII) gives negative responses to both the above colour tests for unsaturation (we are grateful to Dr. E. R. H. Jones for bringing this statement to our notice); here there is no conjugation, and superficially this observation seems to be without parallel. If, however, the assumption is made that, in conjugated systems (C=C—C=O, C=C—NO_2) which cannot be diagnosed by these reactions, a more or less large electromeric shift in the direction of the hetero-atom is vital to the non-production of colour, then the behaviour of (XXII) becomes understandable. For it is known that the αβ-unsaturated ketonic grouping reacts as C=C—C→O, and —C=C·NO_2 will tend to react as C=C—N$\begin{matrix}O\\ \searrow\\ O\end{matrix}$. In the Liebermann–Burchard reaction (concentrated sulphuric acid–acetic anhydride–chloroform) (XXII) may be regarded as salt-forming, *i.e.*, >C:CH·CH_2·$\overset{+}{N}H_2$Ac, which will bring about the condition —C=C—C→N (*a*); whereas in the tetranitromethane reaction the grouping NHAc is in the presence of a powerful oxidising agent, which will therefore be highly kationoid with respect to the nitrogen atom with its unshared electron pair, so that the condition (*a*) will again tend to be attained. On the other hand, in conjugated *ethylenic* linkages in polycyclic hydroaromatic systems there is normally no tendency for such "unilateral" electronic displacements; the electromeric shift is therefore far smaller in magnitude, and the system behaves normally in unsaturation tests.

Experimental.

(Melting points are uncorrected.)

Reduction of Acetoxy-compound (*J*).—The compound (2 g.) was dissolved in boiling amyl alcohol (100 c.c.) and treated with sodium (10 g.), added in small pieces during 80 mins. (more rapidly towards the end of the reaction); a further quantity (50 c.c.) of amyl alcohol was added after the reaction had been in progress for 70 mins. The solution, after being refluxed for a total time of 2 hours, was decomposed with water while still warm. The amyl-alcoholic layers from six such reductions were united, washed with water, and the alcohol removed in steam. The resultant aqueous suspension was shaken with two portions of ether, and the emulsions separated by centrifuging into a sparingly-soluble sodium salt (i) and a neutral fraction (ii).

The ethereal solution (ii) was washed, dried, and evaporated, yielding a residue which rapidly crystallised. The product, which was very sparingly soluble in ether, separated from acetone–methanol in brittle, glassy rods (0·65 g.), which frothed at 135—150° and gave a clear melt at 210—220°, unchanged by further crystallisation. Acetylation of this substance (pyridine–acetic anhydride) gave a product which crystallised from acetone or acetone–methanol in well-formed, prismatic needles, m. p. 195—205° to a paste which cleared at about 250°. The *substance* gave an intense brownish-yellow colour with tetranitromethane in chloroform (Found: C, 78·9; H, 10·5. $C_{30}H_{48}O_3$ requires C, 78·9; H, 10·6%).

Fraction (i) on acidification with acetic acid and extraction with ether yielded the acid (*P*), somewhat sparingly soluble in acetone; it formed flat prisms from methyl alcohol, m. p. 264·5—265·5°. The specific rotation could not be determined in chloroform owing to the low solubility of the acid, or in pyridine because the purest solvent available absorbed yellow light. The diacetyl methyl ester (*Q*), obtained as previously described, was sparingly soluble in methyl alcohol, and crystallised from acetone–methyl alcohol in long flat needles, m. p. 228—229°, $[\alpha]_D^{16°}$ + 63° (*l* = 1, *c* = 3·22 in chloroform) (Found: 73·4; H, 9·9. Calc. for $C_{35}H_{56}O_6$: C, 73·4; H, 9·8%). The pure diacetyl ester was also readily prepared from the crude acid [total yield from 12 g. of (*J*), 2·7 g.].

Diacetyl Methyl Ester Oxide (*R*).—The ester (*Q*) (60 mg.) was dissolved in a chloroform solution of perbenzoic acid (5 c.c. of 0·3N) and kept at 0°. After 11 days the solution and accompanying blank were titrated with 0·1N-thiosulphate (Found: 3·15 c.c. Calc.: 2·10 c.c.). The *oxide*, isolated in the usual manner, separated from acetone in sheaves of fine needles, m. p. 233·5—234·5° (Found: C, 71·5; H, 9·6. $C_{35}H_{56}O_7$ requires C, 71·4; H, 9·6%).

Quantitative Saponification of Diacetyl Methyl Ester (*Q*).—0·4624 G. of the ester was refluxed for 4½ hours with 30 c.c. of 0·3477N-alcoholic potassium hydroxide, and the solution and blank then back-titrated with standard acid (phenolphthalein) (Found: 6·73 c.c. Calc. for $C_{35}H_{56}O_6$, 3 equivs.: 6·97 c.c.). The solution was acidified with acetic acid, and the regenerated acid precipitated with water. After crystallisation from methyl alcohol and then from acetone, it had m. p. 262·5—264° (0·3 g.), undepressed by admixture with an authentic specimen. The ester was also hydrolysed to some extent (1·70 equivs.) after being refluxed for 2½ hours with 6 equivs. of 0·1N-alcoholic alkali; the solution obtained after back-titration was treated with more alcoholic alkali to give a *ca.* 0·05N-solution (400% excess) and refluxed for a further 18 hours, which resulted in a total consumption of 2·73 equivs. The reluctance to undergo complete hydrolysis in the presence of 0·1N, or weaker, alkali may be due either to the carbomethoxyl or to the acetoxyl group.

Diacetyl Acid (*T*).—500 Mg. of the acid (*P*) were dissolved in pyridine (3 c.c.) and heated for 2¾ hours on the steam-bath with acetic anhydride (2 c.c.). The *diacetate*, obtained by precipitation with water, crystallised from aqueous acetone in prisms, m. p. 249—251° after shrinking at 232°, $[\alpha]_D^{19°}$ + 59° (*l* = 1, *c* = 2·565 in chloroform) (Found: C, 72·5; H, 10·0. $C_{34}H_{54}O_6$ requires C, 73·1; H, 9·8%).

Oxidation of Diacetyl Acid.—A solution of the acid (*T*) (500 mg.) in glacial acetic acid (20 c.c.) was treated during 45 mins. at 60—65° with a solution of chromic anhydride (200 mg.) in water (2 c.c.) and acetic acid (10 c.c.). After a further ½ hour, methyl alcohol was added, most of the solvent removed under reduced pressure, and the residue treated with water and extracted with ether–chloroform. The extract was washed with water, 2% sodium hydroxide solution, and again with water. The dried ethereal extract gave no residue on evaporation. Acidification of the alkaline fraction with hydrochloric acid gave the *acid* (*U*), which separated from aqueous acetic acid, aqueous methanol, or aqueous acetone in thin laminæ, m. p. 285—286° after shrinking at 253°. A preparation of the same m. p. was also obtained from another experiment in which the oxidation product was simply precipitated with water and recrystallised; the m. p. was also unchanged after treatment of the acid with pyridine and acetic anhydride [Found (two preparations): C, 71·1, 71·0; H, 9·6, 9·4. $C_{34}H_{54}O_7$ requires C, 71·0; H, 9·5%. $C_{34}H_{52}O_7$ requires C, 71·3; H, 9·2%].

Diacetyl Keto-dihydro Methyl Ester (*S*).—(*a*) Treatment of the foregoing acid with ethereal diazomethane gave a crude product which formed prismatic needles, m. p. 261—264°, from acetone–methanol. Continued recrystallisation yielded the *ester* (*S*) as irregular plates, m. p. 275—277° (Found: C, 71·8; H, 9·8. $C_{35}H_{56}O_7$ requires C, 71·4; H, 9·6%).

(*b*) A solution of the ester (*Q*) (500 mg.) in glacial acetic acid (20 c.c.) was treated with one of chromic anhydride (200 mg.) in water (2 c.c.) and acetic acid (10 c.c.), added during 30—35 mins. at 70—72°. After a further 40 mins., water was added, and the resultant precipitate crystallised repeatedly from acetone–methanol. The product had m. p. 275—277° and gave no depression in m. p. when mixed with the sample obtained by method (*a*).

Diketo-acid (*V*).—A solution of chromic anhydride (80 mg.) in water (1 c.c.) and glacial acetic acid (1 c.c.) was added in one portion to one of the acid (*P*) (180 mg.) in acetic acid (14 c.c.) and water (3 c.c.). After 72 hours at room temperature, water was added, and the filtered and washed *keto-acid* (*V*) recrystallised several times from aqueous alcohol, from which it separated in soft needles, m. p. 192—194° after slight previous sintering, extremely soluble in acetone, methyl and ethyl alcohol, and ethyl acetate (Found: C, 76·7; H, 9·9. $C_{30}H_{46}O_4$ requires C, 76·5; H, 9·9%).

Preparation of Acid (*N*).—To a cold solution of sodium (5 g.) in amyl alcohol (100 c.c.) were added 5 g. of the acetoxy-compound (*J*) and 100 c.c. of amyl alcohol. The whole was refluxed for 1½ hours, the originally clear yellow solution rapidly becoming turbid. The sodium amyloxide was decomposed with water, the amyl alcohol removed in steam, and the aqueous suspension of non-volatile residue extracted twice with ether. Evaporation of the dried ethereal solution gave about 0·5 g. of a partly crystalline residue, which was not identical with (*K*) but has not been further examined. The aqueous fraction, freed from neutral material, was acidified with acetic acid and extracted with ether. Evaporation of the washed and dried solution furnished a crystalline residue, which was recrystallised from aqueous alcohol or acetone. The freshly-prepared acid formed brittle yellow prisms, m. p. 240—241° after sintering at 237°, but the m. p. of a sample which had been kept for some months was only 229—234°. An alcoholic solution of the acid gave a deep greenish-blue colour with ferric chloride [Found (old sample): C, 73·2; H, 9·5; (freshly-prepared sample): C, 73·9; H, 9·5. Calc. for $C_{30}H_{46}O_5$: C, 74·0; H, 9·5%].

Acetyl Methyl Ester (*O*).—Treatment of the above acid with diazomethane in ether–acetone yielded the methyl ester, which crystallised from aqueous methanol in heavy transparent prisms, m. p. 130—145°, apparently containing solvent of crystallisation; one further crystallisation from benzene–ligroin furnished hard yellow rosettes of prismatic needles, m. p. 149—151°. This ester (1 part) was heated at 100° for 2—3 hours with pyridine (3 parts) and acetic anhydride (2 parts). Addition of water precipitated the *acetyl* derivative (*O*), which crystallised from slightly aqueous alcohol in yellow, soft, pearly plates, m. p. 179—180·5°; on rapid cooling of its solutions, the ester showed a tendency to separate as a gelatinous, hair-like mass. The ferric chloride reaction was similar to that given by the free acid, and, like the latter, the m. p. of a sample of the ester which had been kept for some weeks fell considerably (165—170°) [Found (old sample): C, 72·3; H, 9·1; (freshly-prepared sample): C, 72·7; H, 9·2. $C_{33}H_{50}O_6$ requires C, 73·0; H, 9·3%]. *Active hydrogen determination* (*Zerewitinoff*): 3·767 Mg. gave 0·170 c.c. of methane (762 mm., 21°), corresponding to 1·02 active hydrogen atoms. The ester was recovered unchanged after its alcoholic solution had been refluxed with *o*-phenylenediamine for 5 hours or hydroxylamine hydrochloride and sodium acetate for 12 hours.

In a quantitative saponification, 0·6608 g. of the ester was refluxed for 3 hours with 30 c.c. of 0·3462N-alcoholic potassium hydroxide (Found: 6·47 c.c. $C_{33}H_{50}O_6$ requires 7·04 c.c. for 2 equivs.). The regenerated acid, obtained by acidification with acetic acid, was crystallised from aqueous acetone and then from a small volume of absolute methanol; it had m. p. 240·5—242° after slight previous sintering, and gave no depression when mixed with an authentic sample.

Preparation of Substance, $C_{30}H_{42}O_3$ (*W*).—The compound (*K*) (400 mg.) was added to a suspension of phosphorus pentachloride (250 mg.) in ligroin (7·5 c.c.) (b. p. 40—60°). The mixture was refluxed for 2½ hours and then treated with water and extracted with chloroform. The extract was washed with 2% aqueous sodium hydroxide and with water, dried, and evaporated. The residue was crystallised first from aqueous alcohol and then from acetone, from which the *substance* (*W*) separated in stout rods, m. p. 295—297° (decomp.), largely depressed on admixture with the original hydroxy-compound. The Beilstein reaction was negative (Found: C, 80·2; H, 9·3. $C_{30}H_{42}O_3$ requires C, 79·9; H, 9·4%). In different experiments the m. p. was found to vary slightly with alterations in the quantities of reagent and solvent employed. The use of chloroform instead of ligroin gave an oily product from which only a small quantity of low-melting material (m. p. 220—230°) could be isolated.

Experiments with the Lactone, $C_{28}H_{38}O_4$ (*Y*).—(*a*) In numerous minor variations of the original conditions for the preparation of this compound (J., 1939, 755), the original observation was invariably confirmed that the lactone is isolated from the *acid* fraction of the oxidation products of (*K*). To see whether this might not be due to scission of a labile lactone ring of (*Y*) during the working-up of the product, a solution of (*Y*) in chloroform–ether was shaken with portions of 3% aqueous sodium hydroxide, and subsequently with water. Neither the aqueous nor the alkaline washings gave any precipitate on acidification, and on evaporation of the ether–chloroform solution the lactone was recovered unchanged. Since the acid (*Z*) (*q.v.*) shows no tendency to relactonise, it follows that the appearance of (*Y*) in the acid fraction of the oxidation products of (*K*) must be due, as previously surmised, to a loose association between molecules of it and the mixed acids.

The lactone is very sparingly soluble in ether, moderately soluble in hot methyl and ethyl alcohol, and fairly readily soluble in hot ethyl acetate and acetone. The last three solvents were used in the preparation of samples for analysis.

(*b*) The lactone (200 mg.) was added to a cold solution of sodium (200 mg.) in amyl alcohol (4 c.c.). A further 4 c.c. of amyl alcohol were added, and the mixture heated under reflux for 1½ hours. The solution was decomposed with water while still warm, the amyl alcohol removed in steam, the alkaline solution extracted thrice with ether, and then acidified with acetic acid. The precipitate was isolated by means of ether and crystallised from aqueous acetic acid, yielding the *acid* (*Z*) as a micro-crystalline solid, m. p. 247—252° (160 mg.), very soluble in methyl alcohol, ethyl acetate, and acetic acid. It separated as a gel from ethyl acetate–ligroin, but on recrystallisation from aqueous acetic acid formed opaque nodules of tiny needles, m. p. 251—253° (Found: C, 73·4, 73·55; H, 8·7, 8·9. $C_{28}H_{40}O_5$ requires C, 73·6; H, 8·8%). The acid gave no colour with either neutral or acid ferric chloride in alcoholic solution. Esterification with diazomethane in ether–acetone solution gave the methyl ester (*AA*); this separated from aqueous methanol in plates, m. p. 211·5—213°, and gave no depression in m. p. when mixed with an authentic specimen prepared from (*Y*) by hydrolysis with 2N-alcoholic potassium hydroxide.

The ester did not give a coloration in alcoholic solution with ferric chloride; it was recovered unchanged after being heated with pyridine and acetic anhydride at 100° for 4½ hours, and after 5½ hours' refluxing with semicarbazide acetate in alcohol. Hydrolysis of the ester was readily accomplished by refluxing it for 4½ hours with 0·3N-alcoholic potassium hydroxide; the solution, which gave no precipitate on dilution with water, was extracted with ether and then acidified with acetic acid. The precipitated acid (*Z*), on recrystallisation from acetic acid, formed micro-needles, m. p. 249—252°, and 251—253° on admixture with the specimen described above.

Note on Liebermann–Burchard Tests.—In Table II, a substance which gives a clear yellow, or paler, solution, is considered to show a negative reaction. The conditions used throughout the tests were considerably more drastic than the mild conditions which are sufficient to produce positive reactions with steroids and triterpenes containing isolated ethylenic linkages; the tests were not performed on a strictly quantitative basis, but the general procedure was as follows. 1—2 Mg. of the substance were dissolved in 2—3 drops of chloroform, 3—4 drops of acetic anhydride were added, and then 3—5 drops of concentrated sulphuric acid. A considerable difference in sensitivity was noted between substances containing isolated double bonds, *e.g.*, (*P*), (*Q*), (*W*), and those in which a positive reaction was obtained as a result of dehydration at C_2, *e.g.*, (*C*), (*F*), (*J*), (*K*); the latter compounds gave negative results under the mild conditions (low concentrations of acid and substance relative to chloroform) which produce positive reactions with unsaturated steroids.

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