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REINVESTIGATION OF THE ACTION OF HYDROGEN PEROXIDE ON URSOLIC ACID ACETATE†‡

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Abstract—The action of hydrogen peroxide on ursolic acid acetate in boiling acetic acid has been reinvestigated, and the three oxidation products, designated as U_I, U_{II} and U_{III}, have been reisolated. The structure of U_I has been revised to **8a** and that of U_{II} has been established as **11a** on the basis of spectral and chemical evidence. Additional spectral data are provided in support of the structure of U_{III} (**5a**). The stereoelectronic factors responsible for the unusual stability of the epoxide function in U_I towards acid-catalysed rearrangement to the 12-keto-derivative (**9a**) and the facile conversion of the latter to the enol-acetate (**10a**), compared to the normal behaviour of the structurally similar compounds, **8b** and **9d** of the oleanane series, have been rationalised.

Oxidative transformation reactions of the pentacyclic triterpenoids of the α- and β-amyrin series have been the subject of considerable chemical interest since early nineteen thirties. A large number of such reactions which are mostly initiated by the 12,13-double bond in the aforesaid triterpenoid skeletons has been studied by several groups of workers.[1-11] In 1946 Jeger *et al.*[9] studied the action of hydrogen peroxide on ursolic acid acetate (I) in hot glacial acetic acid and reported the isolation of three crystalline compounds which, in the present discussion, are designated as U_I, $C_{32}H_{48}O_5$, m.p. 280–82°, $[\alpha]_D$ + 51° ($CHCl_3$), U_{II}, $C_{32}H_{50}O_5$, m.p. 279° $[\alpha]_D$ + 30° ($CHCl_3$) and U_{III}, $C_{32}H_{50}O_5$, m.p. 321° [methyl ester, $C_{33}H_{52}O_5$, m.p. 246–50°, $[\alpha]_D$ + 35° ($CHCl_3$)]. These authors, however, did not assign any structure to U_{II}, but proposed, mainly on the basis of analytical data and some chemical evidence, the partial structures **2** and **3** for U_I and U_{III}, respectively. Extension of these partial formulations in the subsequently established ursane skeleton gave the complete structure **4** and **5a** for U_I and U_{III}, respectively. Later in 1957, Simonsen and Ross[12] referred to this work in the review on terpenoids and strongly disapproved the formulation **4** for U_I. They suggested the structures **6** or **7** for U_I without, however, providing any positive evidence in support of their proposition. This has prompted us to undertake a thorough reinvestigation of this work. Accordingly, U_I, U_{II} and the methyl ester of U_{III} were reisolated and the reported molecular formulae of the compounds were confirmed by their mass spectrometrically derived molecular weights of 512, 514 and 528 respectively. In this communication we report spectral and chemical evidences which necessitated revision of the structure of U_I, and established those of U_{II} and U_{III}.

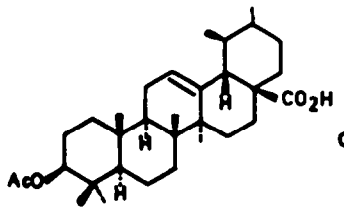
The IR spectrum of U_I shows bands for acetoxy (1725 and 1240 cm^{-1}) and epoxide (872 cm^{-1}) function and an intense band at 1765 cm^{-1} which clearly indicates the presence of a γ-lactone rather than an ϵ-lactone as required by the formulation **4**. Examination of Dreiding model also shows that construction of **4** is sterically impossible. The most decisive evidence against the structure **4** was provided by the PMR spectrum of U_I showing usual signals for seven C-methyls (δ 0.82–1.23), the C-3 acetoxy function (δ 2.06, 3 H, s) and the C-3 methine proton (δ 4.56, 1 H, m). The spectrum, however, shows no other downfield signal below 4 ppm which could be ascribed to a methine proton geminal to the O atom of a lactone function, and, instead, contains two one-proton signals at δ 2.95 (d, J 4 Hz) and 3.10 (br. signal, Wh/2 = 4 Hz). The chemical shifts and the splitting patterns of these protons are strikingly similar to those reported[1,7c,13-15] for the vicinal *cis*-oriented hydrogens in several oxirane systems. The presence of two such protons, the absence of any lactonic methine proton and the characteristic γ-lactone absorption in the IR spectrum of U_I thus completely invalidated the formulation **4** derived by extension of the partial structure **2** proposed for the compound by Jeger *et al.* These observations, at the same time, also ruled out the alternative structures **6** and **7** for U_I suggested by Simonsen and Ross, but are best explained in terms of the structure **8a**. The formation of U_I as formulated above finds strong analogy among structurally similar compound **8b** obtained by photo-oxidation of oleanolic acid.[7] The two one-proton signals at δ 2.95 and 3.10 have been assigned, respectively, to the C–12H and C–11H of U_I. The observed Wh/2 (4 Hz) of the signal for C–11H supports the α-orientation of the epoxide function, which is also preferred on steric grounds.

The mass fragmentations of U_I exhibit striking similarity with those of the isomeric epoxy-γ-lactone **8b** of the oleanane series. Besides the molecular ion (*m/e* 512), the significant peaks at *m/e* 468, 277, 263, 249, 235, 218, 217, 205, 204, 203, 189 and 175 appearing in the MS of U_I can be best rationalised (Scheme 1) in terms of the structure **8a**.

Further evidence in support of the structure **8a** for U_I was provided by the following transformation reactions of the compound. Acid-catalysed hydrolysis of U_I with 6 N aqueous ethanolic sulphuric acid for 10 hr under

†A preliminary account of this work was presented in the Symposium on Isoprenoids: Chemistry and Applications held at Malti-Chem. Research Centre, Nandesari, Baroda, 1975, Abstracts of Papers, B 23.

‡Dedicated to Professor (Mrs.) Asima Chatterjee, Head of the Department of Chemistry, University of Calcutta, on the occasion of her 60th birthday.



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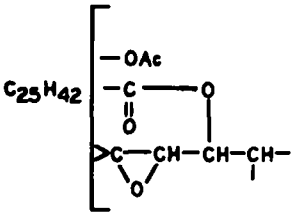

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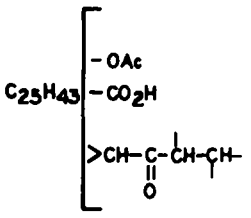

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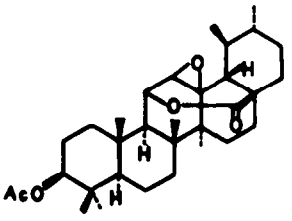

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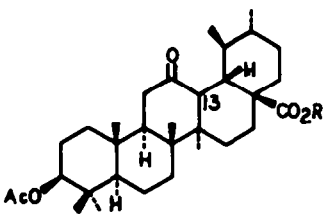

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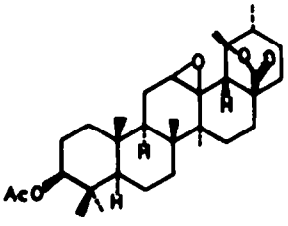
a: R = H; b: R = Me



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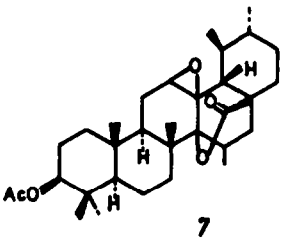

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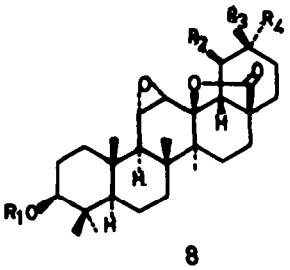
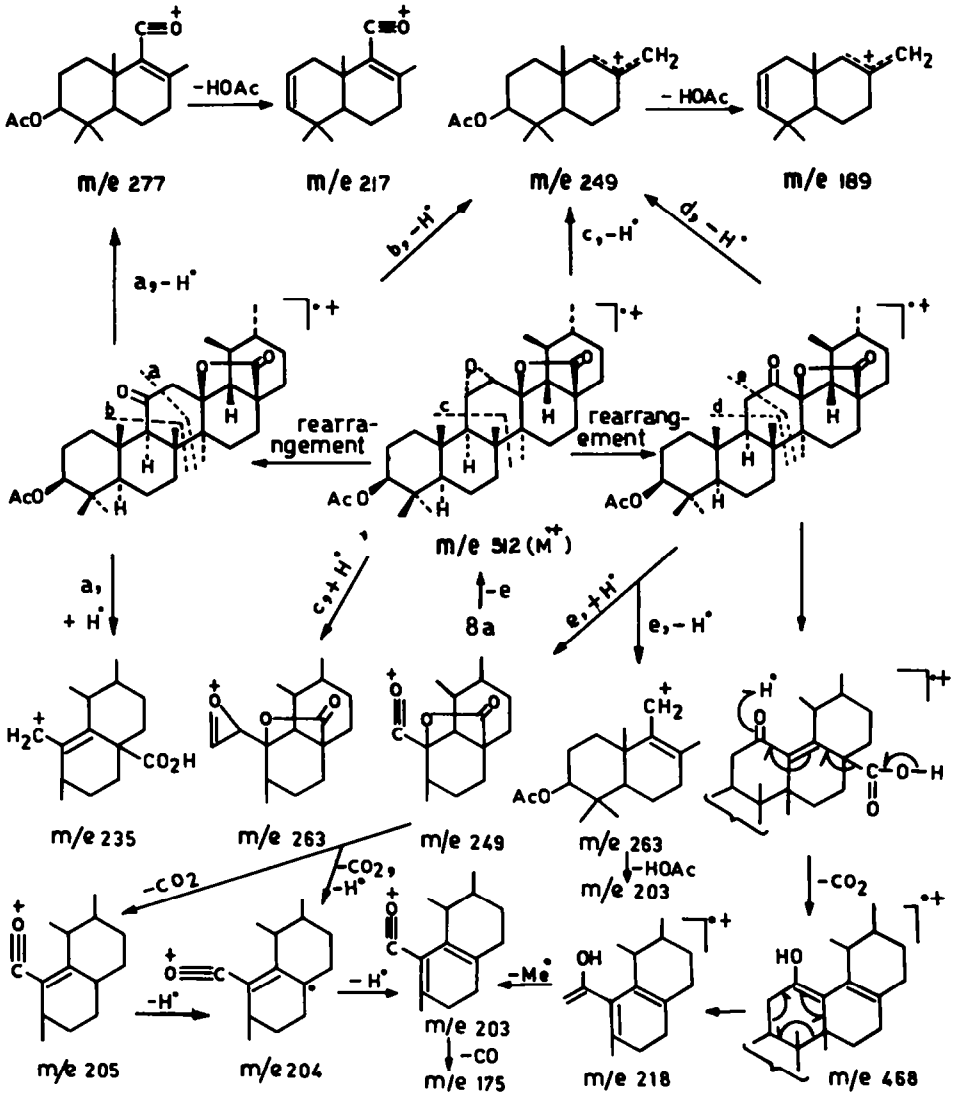


8

a: R_1 = Ac, R_2 = R_4 = Me, R_3 = H
b: R_1 = Ac, R_2 = H, R_3 = R_4 = Me
c: R_1 = R_3 = H, R_2 = R_4 = Me

refluxing conditions followed by acetylation of the reaction products afforded, on chromatographic resolution, besides an appreciable amount of U_I, a new compound $C_{34}H_{50}O_6$ ($M^{+\cdot}$ 554), m.p. 288°. The IR spectrum of the latter shows bands at 1720 and 1240 (–O–C–CH_3) and

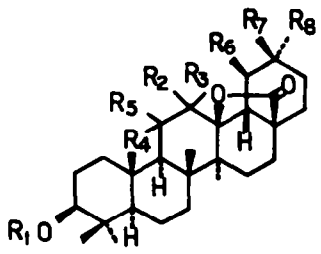
1755 (γ-lactone) cm^{-1}. The PMR spectrum of the compound exhibits, besides the usual signals for seven C-methyls (δ 0.88–1.23), the C-3 acetoxy (δ 2.05, 3 H, s) and the C-3 methine (δ 4.53, 1 H, m) protons, two additional signals at δ 2.15 (3 H, s) and 5.85 (1 H, broad signal with fine splitting, Wh/2 = 3 Hz) which strongly suggest the presence, in its molecule, of an enol-acetate function of the type –CH–CH=C(OAc)–C– or –CH–C(OAc)=CH–C–. This is further supported by the fact that acid-hydrolysis of the above acetate gave the corresponding deacetyl derivative, $C_{30}H_{46}O_4$ ($M^{+\cdot}$ 470), m.p. 283–85°, which shows in its IR spectrum bands at 3450 (–OH), 1765 (γ-lactone) and 1698 cm^{-1}, the last one being attributed to a 6-membered cyclic ketone. Moreover, the above deacetyl derivative has been readily converted to the aforesaid enol-acetate by the action of acetic anhydride and pyridine. These observations thus firmly establish the presence of an epoxide function at C-11 and C-12 in U_I and are intelligible in terms of a rearrangement of the latter to either a 12-keto (**9a**) or an 11-keto-(**9b**) γ-lactone derivative, which on acetylation gives the enol-acetate **10a** or **10b**. The position of the enol-acetate function in the latter and that of the keto group in the former has been settled by comparison of their various spectral properties with those of the corresponding compounds of the oleanane series. For this purpose an authentic sample of 3β - acetoxy - 12α - hydroxy - olean - 28 - oic - 13(28) - lactone (**11b**) was oxidised with



Scheme 1.

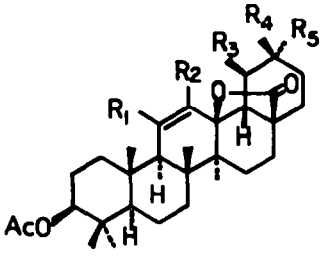
CrO_3/Py to give the corresponding 12-keto-compound **9e**. The latter upon refluxing with Ac_2O/Py was converted for the first time to a compound, $C_{34}H_{50}O_6$ ($M^{+\cdot}$ 554), m.p. 255°, which from its various spectral data was shown to have the enol-acetate structure **10c**, isomeric with that derived from U_I. Acid-hydrolysis of either **10c** or **9e** gave the 12-keto-compound, $C_{30}H_{46}O_4$ ($M^{+\cdot}$ 470), m.p. 304° identical with **9d**, obtained by acid-catalysed rearrangement of **8b**. As expected, refluxing **9d** with Ac_2O/Py regenerated **10c**. The olefinic proton of the latter appears at δ 5.88 having identical splitting pattern (Wh/2 = 3 Hz) with that of the enol-acetate derived from U_I. By analogy, the structures of the U_I-derived enol-acetate and its deacetylderivative were postulated as **10a** and **9a** respectively. The virtual identity of the mass spectra of **9a** and **10a** with those of **9d** and **10c**, respectively, is also in accord with this conclusion. Further, the striking resemblance of the CD curve of **9a** to that of **9d**, as well as to that of **9e**,[16,17] all showing characteristic negative Cotton effect supports the location of the keto group in **9a** and hence the enol-acetate function in **10a** at C-12. All these observations in turn finally establish the structure of U_I as 3β - acetoxy - 11α,12α - epoxyursan - 28 - oic - 13(28) - lactone (**8a**).

It may be noted that U_I(**8a**) and the analogous compound **8b** of the oleanane series exhibit a remarkable difference in reactivity of their epoxide function towards acid. The latter shows normal behaviour of an epoxide derivative undergoing smooth acid-catalysed (refluxing with 6 N aqueous ethanolic H_2SO_4 for only 20 mn) rearrangement to the 12-keto compound **9d**, while the former under similar reaction conditions is mostly changed to its deacetyl derivative (**8c**), $C_{30}H_{46}O_4$ ($M^{+\cdot}$ 470), m.p. 296°, ν_{max} 3300 (OH) and 1775 (γ-lactone) cm^{-1}, convertible to U_I on reacetylation. Working under the conditions of Jeger *et al.*[9] (refluxing a solution of U_I in MeOH/C_6H_6/conc HCl for 3.5 hr) followed by acetylation of the total reaction products, we could, however detect by means of tlc the presence of the enol-acetate **10a** in extremely poor yield. The initial formation in very poor yield of the 12-keto compound **9a** from which **10a** is obtained as above possibly escaped the notice of the previous workers. Even by increasing the concentration of the acid and the reaction period (refluxing a solution



9

a: $R_1=R_4=R_5=R_7=H$; $R_6=R_8=Me$; $R_2,R_3=O$
b: $R_1=R_2=R_3=R_7=H$; $R_6=R_8=Me$; $R_4,R_5=O$
c: $R_1=Ac$; $R_4=R_5=R_7=H$; $R_6=R_8=Me$; $R_2,R_3=O$
d: $R_1=R_4=R_5=R_6=H$; $R_7=R_8=Me$; $R_2,R_3=O$
e: $R_1=Ac$; $R_4=R_5=R_6=H$; $R_7=R_8=Me$; $R_2,R_3=O$



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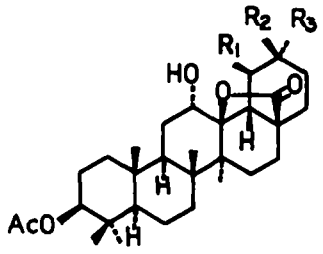


11

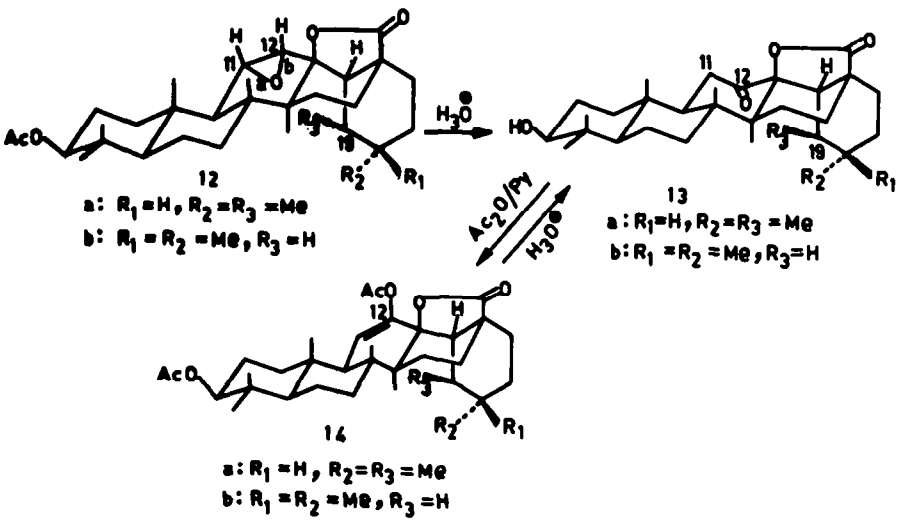
a: $R_1=R_4=H$; $R_2=OAc$; $R_3=R_5=Me$
b: $R_1=OAc$; $R_2=R_4=H$; $R_3=R_5=Me$
c: $R_1=R_3=H$; $R_2=OAc$; $R_4=R_5=Me$

a: $R_1=R_3=Me$; $R_2=H$
b: $R_1=H$; $R_2=R_3=Me$

of U_I in 6 N ethanolic H_2SO_4 for 10 hr) the maximum yield of **9a** that could be obtained is about 25%, the rest being the deacetyl-U_I (**8c**). A further interesting observation is that the 12-keto compound **9a** is smoothly converted (on slight warming with Ac_2O/Py) to the enol-acetate **10a**, whereas the analogous 12-keto compound **9d**, derived from either **8b**, or **9e** gave the enol-acetate derivative **10c** only after prolonged reflux with Ac_2O/Py. The stereo-electronic factors responsible for the observed difference in the reactivities of the epoxide function in **8a** and **8b** and that of the 12-keto group in **9a** and **9d** (and hence of **9e**) may be explained by examination of the conformations of **8a**, **8b**, **9a**, **9d** and of the enol-acetates **10a**, and **10c** represented as **12a**, **12b**, **13a**, **13b** and **14a** and **14b** respectively, (Scheme 2) constructed on the basis of their Dreiding models.

In both **12a** and **12b** cleavage of bond (a) with concomitant hydride shift from C-12 and usual hydrolysis of 3-acetoxy function leads to the formation of the corresponding 12-keto derivatives **13a** and **13b**. The alternative cleavage of the bond (b) to give the corresponding 11-keto compound is presumably inhibited due to the destablization of the incipient carbonium ion to be formed at C-12 by the electron-withdrawing inductive effect of the lactone function at C-13. Now, it may be seen that the OH group in the incipient carbonium ion at C-11 formed by cleavage of the bond (a) in **12a** would be under severe steric interaction with C-19-Me group, raising the energy of the transition state too high. As a result, the reaction leading to the formation of **13a** from **12a** is unusually suppressed. On the other hand, no such steric factor is operative in **12b** ($R_3=H$) and consequently it rearranges smoothly to **13b**. The latter should again behave as a compound containing a normal keto-methylene group and as expected forms the enol-acetate **14b** only under refluxing conditions. The situation with **13a**, on the other hand, is altogether different. The 12-keto and the 19-Me groups in **13a** are again under severe steric strain which increases the thermodynamic instability of the compound. The driving force for the facile formation of the enol-acetate **14a** from **13a** is obviously the release of the above steric strain.

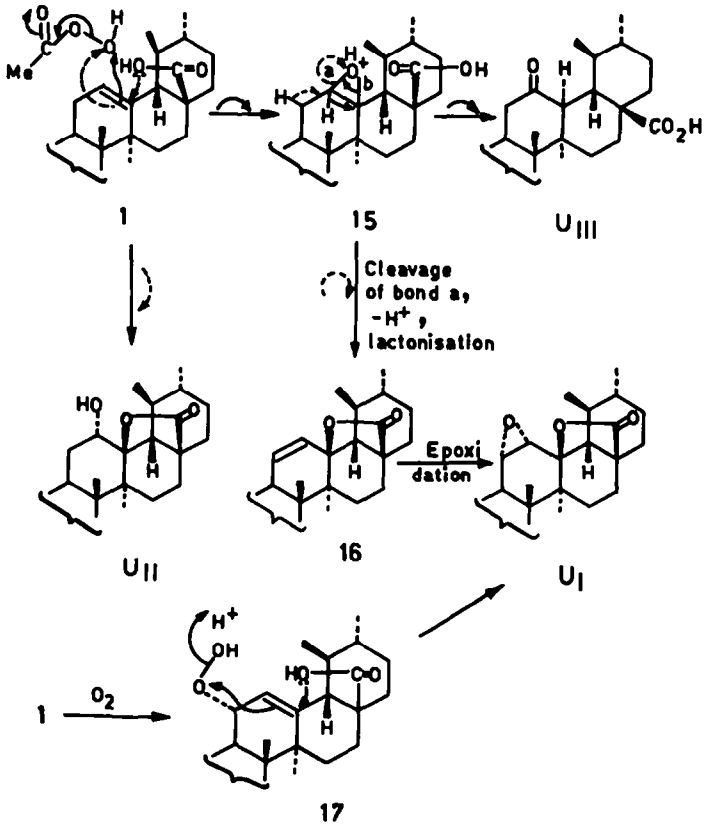
U_{II} shows in its IR spectrum bands for acetoxy function (1725 and 1250 cm^{-1}), γ-lactone (1775 cm^{-1}) and OH group (3300 cm^{-1}). The presence of an OH function in U_{II} is also supported by the formation of an acetyl derivative, $C_{34}H_{52}O_6$ ($M^{+\cdot}$ 556), m.p. 302°, ν_{max} 1775, 1740 and 1250 cm^{-1}. The PMR spectrum of U_{II} shows, besides the usual signals for seven C-Me (δ 0.86–1.22) and the secondary acetoxy function at C-3 (δ 2.05, 3 H, s and 4.50, 1 H, m), the presence of a one-proton broad signal at δ 4.03 (Wh/2 = 8 Hz) attributable to a carbinyl proton geminal to an axial OH group. The OH proton appears at δ 3.70 disappearing on deuterium exchange. The mass spectral fragmentations of U_{II} exhibit striking similarities with those of 3β - acetoxy - 12α - hydroxy -



Scheme 2.

olean - 28 - oic - 13(28) - lactone (**11b**). These observations are thus consistent with an analogous structure **11a** for U_{II}, the seterochemistry of the OH group being assigned on the basis of similar splitting pattern of the signals due to the hydroxy methine protons in U_{II} and **11b**.

More compelling evidence in support of the structure **11a** for U_{II} has been secured from the following reactions and its chemical correlation with U_I. Oxidation of U_{II} with CrO_3/Py gave a compound, $C_{32}H_{48}O_5$ ($M^{+\cdot}$ 512), m.p. 274°, which shows in its IR spectrum bands for acetoxy group (1725 and 1250 cm^{-1}), γ-lactone (1765 cm^{-1}) and a 6-membered cyclic keto-carbonyl (1705 cm^{-1}) function. The MS of the compound is strikingly similar to that of the 12-keto-compound **9e** of the oleanane series. These together with its characteristic PMR spectral data strongly support the structure **9c** for the compound. This is further corroborated by the fact that the physical constants of this compound compare excellently with those reported for 3β - acetoxy - 12 - oxo - ursan - 28 - oic - 13(28) - lactone prepared by Mezzetti *et al.*[2] Although a direct comparison could not



Scheme 3.

be made, it appears that the two compounds are identical in all respects. This is also borne out by the fact that on treatment with Ac_2O/Py, **9c** gave the same enol-acetate **10a** derived from U_I. Alternatively, acid-hydrolysis of **9c** or **10a** gave the identical 12-keto-compound **9a** obtained from U_I. These chemical correlations thus firmly establish the structure of U_{II} as 3β - acetoxy - 12α - hydroxy - ursan - 28 - oic - 13(28) - lactone (**11a**).

U_{III} was isolated as its methylester. The IR (ν_{max} 1725, 1700 and 1250 cm^{-1}) and PMR [δ 0.72–0.98 (7-C-methyls); 2.05, 3 H, s and 4.5, 1 H, m (–CH–OCOCH$_3$); 2.43, 2 H, m and 2.66, 1 H, br signal (–CH–CH$_2$–C(=O)–CH–CH–); 3.73, 3 H, s (–CO_2Me)] spectral data of the methyl ester of U_{III} recorded for the first time, when considered along with its physical constants strongly support its identity with 3β - acetoxy - methylketodihydroursolate (**5b**) prepared independently by Ruzicka *et al.*,[10] and Manson *et al.*[11b] Consequently, the structure of U_{III} should be represented as **5a** as postulated by Jeger *et al.*,[9] the stereochemistry of C-13 H being tentatively assigned as α on the basis of its mode of formation.

Finally it would be instructive to comment on the mechanism of formation of U_I, U_{II} and U_{III}. Although the precise mechanism is still obscure, it appears quite clear that the formation of all the three compounds is the result of an initial reaction of peracetic acid (formed *in situ*) with the 12,13-double bond of ursolic acid acetate. A plausible mechanistic rationale as depicted in Scheme 3 may be envisaged as a working hypothesis. The reaction of peracetic acid on the 12,13-double bond of ursolic acid acetate may directly lead to the formation of U_{II}, the γ-lactone function being formed by the nucleophilic participation of the C-17 carboxylic group. Alternatively, the same reaction may result in the initial formation of a 12,13-epoxy-derivative **15**, with the carboxyl group remaining intact. Acid-catalysed rearrangement of **15** [cleavage of bond (b)] with concomitant hydride shift forms U_{III}. Opening of the epoxide function in **15** by the alternative cleavage of bond (a) followed by lactonisation may give the intermediate, **16**, which on subsequent epoxidation may lead to the fomration of U_I. The formation of the latter through the intermediacy of the hydroperoxide **17** (Scheme 3), analogous to those conceived by Corey *et al.*[1] in the autooxidation of α- and β-amyrins, and by Kitagawa *et al.*[7a–c] in the photochemical transformation of oleanolic acid, seems unlikely in the present case in view of the reaction conditions, viz. boiling acetic acid, unfavourable for the availability of the triplet oxygen necessary.

EXPERIMENTAL

M.ps were determined in a Köfler block and were uncorrected. Unless otherwise stated, IR spectra were run in Nujol mulls in Beckman Infrared Spectrophotometer (Model 20). PMR spectra were recorded in a 60 MZ instrument in $CDCl_3$ solution using TMS as internal standard. Mass spectra were run in an AEI MS 9 instrument equipped with a direct inlet system and operating at 70 eV. Metastable peaks are indicated by m* and r.i. stands for relative intensity. Silica gel (60–100 mesh) was used for column chromatography and silica gel G for tlc performed at room temp. (25–35°). All analytical samples were routinely dried over P_2O_5 at 55–138° depending on the m.ps of the compounds for 24 hr *in vacuo*. Anhydrous Na_2SO_4 was used for drying organic solvents and petrol used had b.p. 60–80°.

Formation of U_I (**8a**), *U_{II}* (**11a**) *and methylester of U_{III}* (**5b**). Pure ursolic acid acetate (0.9 g for each set of reactions) m.p. 282° was dissolved in glacial HOAc (27 ml). A mixture of 30% H_2O_2 (5.7 ml) and glacial HOAc (5.7 ml) was prepared from which 7.8 ml were added uniformly to the ursolic acid acetate soln during 15 min. After 2 hr the remaining soln was added during 10 min and the reaction was allowed to proceed for another hr. All these operations were done at a temp. of 100–105° with continuous stirring. The mixture was then largely diluted (75 ml). The solid separated was collected, washed with water and crystallised from MeOH to give U_I (0.15 g), m.p. 280°; R_f 0.65 in petrol–EtOAc (3:1) as the developer; *m/e* (r.i.): 512 ($M^{+\cdot}$, 31.8), 497 (15.9), 494 (7.9), 484 (9.5), 468 (7.8), 452 (11.1), 437 (7.9), 316 (10.3), 300 (15.9), 278 (16.6), 277 (72.2), 263 (61.9), 250 (19.0), 249 (27), 235 (18.3), 232 (19), 231 (20.6), 218 (15.9), 217 (39.7), 206 (13.5), 205 (49.2), 204 (53.9), 203 (58.7), 190 (34.9), 189 (100), 188 (38), 187 (28.6), 175 (34.1), 163 (23), 161 (22.3), 159 (21.4), 147 (45.3), 135 (57), 134 (23.9), 133 (36.5), 123 (27), 121 (47.7), 119 (63.5), 109 (42) and 105 (42); m* *m/e* 482.5, 399, 384, 371.5, 228.5, 179, 166.5, 139, 152, 89, 77 and 75.

The combined mother liquor after crystallisation of U_I was evaporated to dryness and the residue was methylated with CH_2N_2 in the usual manner. The methylated product was chromatographed. The petrol-C_6H_6 (3:1) eluate furnished after evaporation a further amount (0.005 g) of U_I. The concentrated petrol–C_6H_6 (1:1) eluate afforded a solid which crystallised from MeOH to give methylester of ursolic acid acetate (0.01 g). Evaporation of the early fractions of the C_6H_6–$CHCl_3$ (4:1) eluate yield a solid which crystallised from MeOH to give methylester of U_{III} (0.05 g), m.p. 246–50°; R_f 0.45 in petrol–EtOAc (3:1) as the developer. The later fractions of C_6H_6–$CHCl_3$ (4:1) eluate, on removal of solvent, gave a solid which crystallised from acetone–methanol to give U_{II} (0.12 g), m.p. 275°; R_f 0.5 in petrol–EtOAc (2:1) as the developer; *m/e* (r.i.): 514 ($M^{+\cdot}$ 14), 496 (26.2), 454 (2.5), 436 (9.8), 300 (33.7), 251 (20.3), 250 (25.6), 249 (25.6), 246 (26.2), 234 (27.9), 206 (25), 205 (100), 204 (48.8), 203 (24.4), 190 (26.1), 189 (83.7), 175 (15.1), 161 (25.6), 147 (19.1), 136 (26.7), 135 (36), 134 (18), 133 (17.4), 123 (23.3), 121 (29.1), 119 (22.1), 109 (25.5), 107 (26.7) and 105 (14.5); m* *m/e* 384, 212, 175.2, 170.7, 166.5, 152.8, 143.5 and 105.5.

Acid catalysed hydrolysis of U_I (**8a**)

(i) A soln of U_I (0.1 g) in C_6H_6 (1 ml) and MeOH (4 ml) was refluxed with conc HCl (0.5 ml) for 3.5 hr according to the method of Jeger *et al.*[9] The residue obtained after removal of solvent under reduced pressure was diluted with water, extracted with ether and dried. Evaporation of ether left a residue which was treated with Ac_2O/Py and the mixture was warmed on a water bath for 30 min. The mixture was diluted with water and the liberated solid was collected and dried. Tlc of this solid showed that it was a mixture of mostly unchanged U_I and traces of **10a**, R_f 0.6 in petrol–EtOAc (4:1) as the developer. The mixture was chromatographed. Petrol–EtOAc (10:1) eluate gave U_I (0.09 g), and petrol–EtOAc (9:1) eluate on evaporation afforded only a trace of **10a**.

(ii) A mixture of U_I (0.14 g), 6 N H_2SO_4 (15 ml) and abs EtOH (20 ml) was refluxed for 10 hr over a low flame. After cooling, EtOH was removed under reduced pressure. The residue after dilution with water was extracted with ether and dried. Removal of solvent left a residue which was acetylated with Ac_2O/Py as above, and the mixture was worked up, also in the same manner. Similar chromatography of the reaction products afforded U_I (0.1 g) and **10a** (0.03 g) crystallised in fine needles from petrol–EtOAc (6:1), m.p. 288°. (Found: C, 73.60; H, 9.05. $C_{34}H_{50}O_4$ requires: C, 73.65; H, 9.03%). *m/e* (r.i.) 554 ($M^{+\cdot}$, 8.6), 512 (8.6), 510 (6.4), 497 (7.0), 468 (6.4), 460 (5.4), 291 (45.2), 250 (9.7), 249 (52.7), 221 (12.9), 204 (21.5), 203 (100), 189 (10.8), 175 (14), 135 (23.7), 133 (15.1), 121 (19.4) and 119 (19.4); m* *m/e* 183.6, 173.4 and 141.8.

(iii) A mixture of U_I (0.04 g), 6 N H_2SO_4 (3 ml) and abs EtOH (6 ml) was refluxed for 20 min over a low flame. EtOH was removed under reduced pressure. The residue was diluted with water, extracted with $CHCl_3$ and dried. The $CHCl_3$ layer was concentrated and chromatographed. Petrol–EtOAc (10:1) eluate

gave unchanged U_I (0.005 g). Petrol–EtOAc (7:1) eluate furnished **8c** (0.033 g) crystallised from the same solvent mixture (5:1), m.p. 296°, R_f 0.35 in petrol–EtOAc (3:1) as the developer. (Found: C, 76.50; H, 9.81. $C_{30}H_{46}O_4$ requires: C, 76.60: H, 9.79%).

0.01 g of **8c** was acetylated with Ac_2O/Py in the usual manner to give 0.009 g of U_I.

Acid-catalysed hydrolysis of **10a** *to* **9a** *and its regeneration from* **9a**. A mixture of **10a** (0.05 g), 6 N H_2SO_4 (5 ml) and abs EtOH (5 ml) was refluxed for 30 min over a low flame. After cooling, EtOH was removed under reduced pressure. The residue was diluted with water, extracted with ether and dried. Removal of solvent left a residue which crystallised from petrol–EtOAc (4:1) to give **9a** (0.045 g), m.p. 283–85°; R_f 0.3 in petrol–EtOAc (3:1) as the developer (Found: C, 76.53; H, 9.81. $C_{30}H_{46}O_4$ requires: C, 76.60; H, 9.79%). c.d. (MeOH): $[\theta]_{309} - 1954$ and $[\theta]_{228} + 2077$.

Compound **9a** (0.01 g) was treated with 1 drop of pyridine and Ac_2O (1 ml). The mixture was warmed on a water bath for 30 min. Usual work up gave **10a** (0.009 g).

Chromic acid oxidation of **11b** *to* **9e** *and the formation of* **10c** *from* **9e**. Compound **11b** (0.1 g) was oxidised by CrO_3 to **9e** (0.087 g), m.p. 270° following the method of Kitagawa *et al.*[7c] 0.05 g of **9e** was treated with Ac_2O (2 ml) and pyridine (0.5 ml) and the mixture was gently refluxed for 3 hr. After cooling, the product was worked up in the same manner as in the case of **10a**. The crude solid obtained was chromatographed. Petrol–EtOAc (12:1) eluate gave unchanged **9e** (0.03 g). Petrol–EtOAc (11:1) eluate, on evaporation, gave **10c** (0.015 g) crystallised from the same solvent mixture (8:1), m.p. 255°; R_f 0.6 in petrol–EtOAc (4:1) as the developer. (Found: C, 73.59; H, 9.00. $C_{34}H_{50}O_6$ requires: C, 73.65; H, 9.03%). *m/e* (r.i.): 554 ($M^{+\cdot}$, 2.0), 512 (7.3), 510 (2.0), 497 (7.0), 468 (8.0), 466 (5.7), 302 (4.7), 301 (18), 292 (6.0), 291 (28.7), 250 (4.7), 249 (23.4), 231 (17.3), 221 (15.7), 205 (8.7), 204 (20.7), 203 (100), 189 (17.3), 175 (26.6), 135 (14.0), 133 (11.3) and 121 (13.3).

Acid-catalysed hydrolysis of **10c** *and* **9e** *to* **9d** *and the regeneration of* **10c** *from* **9d**. A mixture of **10c** (0.04 g), 6 N H_2SO_4 (3.6 ml) and abs EtOH (4.6 ml) was refluxed for 20 min over a low flame. It was then worked up as in the case of acid-catalysed hydrolysis of **10a** to give **9d** (0.038 g) crystallised from petrol–EtOAc (3:1), m.p. 304° (Found: C, 76.53; H, 9.83. $C_{30}H_{46}O_4$ requires: C, 76.60; H, 9.79%). CD (MeOH): $[\theta]_{325} + 297.3$, $[\theta]_{289} - 594.6$ and $[\theta]_{225} + 3642$.

A mixture of **9e** (0.01 g), 6 N H_2SO_4 (1 ml) and abs EtOH (1.5 ml) was refluxed for 30 min. The mixture was then worked up as above to give **9d** (0.009 g).

A soln of **9d** (0.02 g) in pyridine (0.1 ml) and Ac_2O (1 ml) was gently refluxed for 3 hr. After cooling, the mixture was worked up in the same manner as in the case of acetylation of **9a**. The crude product was chromatographed. 3 - O - acetyl - 12 - keto - oleanolic lactone (0.012 g) was obtained from the petrol–EtOAc (12:1) eluate. Further elution of the column with petrol–EtOAc (11:1) gave **10c** (0.006 g).

Acetylation of U_{II}. A mixture of U_{II} (0.02 g), pyridine (0.1 ml) and Ac_2O (1 ml) was warmed on a water bath for 30 min and then kept overnight. It was then worked up in the usual manner to give 12-O-acetyl-U_{II} (0.018 g), crystallised from petrol–EtOAc (5:1), m.p. 302°. (Found: C, 73.29; H, 9.40. $C_{34}H_{52}O_6$ requires: C, 73.38; H, 9.35%).

Chromic acid oxidation of U_{II} *and the formation of* **9c**. To a stirred soln of U_{II} (0.1 g) in acetone (25 ml) was added dropwise CrO_3 soln (1.5 ml) (composition: CrO_3, 1.33 g; conc H_2SO_4, 1.5 ml; H_2O, 3.8 ml) at room temp. and the mixture was kept stirred for further 20 min and then diluted with water. The white ppt that appeared was collected by filtration, dried and chromatographed. Petrol–EtOAc (10:1) eluate on evaporation, gave **9c** (0.07 g) crystallised from petrol–EtOAc (4:1), m.p. 274°. (Found: C, 74.95; H, 9.40. $C_{32}H_{48}O_5$ requires: C, 75.00; H, 9.38%), *m/e* (r.i.): 512 ($M^{+\cdot}$, 39), 468 (18.3), 453 (7.3), 452 (7.3), 250 (19.5), 249 (100), 248 (24.4), 218 (27.3), 205 (28.5), 204 (34.2), 203 (24.4), 190 (12.2), 189 (62.2), 175 (24.4), 161 (13.4), 147 (17.1), 135 (19.5), 134 (12.2), 133 (12.2), 121 (20.7) and 119 (22); m* *m/e* 468, 428.5, 399, 248, 196.5, 165.5, 143.5 and 121.

Formation of **10a** *from* **9c**. A mixture of **9c** (0.02 g), Ac_2O (1 ml) and pyridine (0.5 ml) was warmed on a water bath for 30 min and kept overnight at room temp. Usual work up of the reaction mixture gave **10a** (0.018 g).

Acid-catalysed hydrolysis of **9c**. A soln of **9c** (0.015 g) in 6 N H_2SO_4 (1.5 ml) and abs EtOH (2 ml) was refluxed for 30 min over a low flame. The mixture was worked up as in the case of the hydrolysis of **10c** to give **9a** (0.013 g).

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