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THE STRUCTURE AND CHEMISTRY OF HIRSUTIC ACID*

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Abstract By a necessary combination of chemical and X-ray studies the structure and stereochemistry of the sesquiterpenoid acid, hirsutic acid C ($C_{15}H_{20}O_4$) from *Stereum hirsutum* have been established as shown in I. An unusual rearrangement involving solid state X-irradiation has been discovered and evaluated.

THE mould *Stereum hirsutum* elaborates a number of acidic metabolites, some of which have antibiotic properties. The most abundant of these, hirsutic acid C, m.p. 180° $[\alpha]_D$ +116° was first described by Heatley *et al.* in 1947 and the molecular formula $C_{15}H_{20}O_4$ assigned.[1] It therefore seemed possible that hirsutic acid was a member of the sesquiterpenoid family. Although several strains of *S. hirsutum* were examined in attempts to reisolate this acid, in our hands cultures of *S. hirsutum* have not so far yielded terpenoid material. However through the generosity of Dr. N. G. Heatley we have been able to examine the chemistry of hirsutic acid C‡ and on the basis of the following evidence, the structure and stereochemistry (I(R=H) may be assigned.

Hirsutic acid and its methyl ester showed only end absorption in the UV spectrum. The acid exhibited IR absorption at 3520 (OH) 3200 (CO_2H) 1700 (>C=O of CO_2H) 1655 (C=C) and 890 (= CH_2) cm^{-1}. The NMR spectrum of methyl hirsutate confirmed the presence of two Me groups, a secondary OH group and a double bond, as follows. Two distinct tertiary Me groups appeared as singlets at τ 8·66 and 8·97 (respectively) the characteristic doublets of exocyclic methylene at τ 4·72 and 5·00 and a multiplet at τ 5·40 suggested the C—H proton of a secondary alcohol. The relationship between the latter proton and the exomethylene hydrogens appeared to be 1 : 3, as long range couplings J = 2·1, 2·7 c/s were found for H_a, H_c, (See II) and H_b, H_c respectively. H_c (τ 6·55) in turn was coupled to H_d (J H_c, H_d = 1·9 c/s). The chemical shift of the H_d doublet indicated that this proton was subtended from a carbon at an epoxide terminus. Partial structure II accommodates the above spectroscopic data.

The formula $C_{15}H_{20}O_4$ was next confirmed by mass spectrometry. In particular methyl hirsutate showed in addition to a strong molecular ion peak (M^+, m/e 278) the loss of Me, OH, and carbomethoxy groups [m/e 263 (M-15); 260 (M-18), 219 (M-44 + 15); and 201 (M-59 + 18)].

The presence of the secondary OH group and its relationship to the exocyclic

* Preliminary Communication: F. W. Comer, F. McCapra, I. H. Qureshi, A. I. Scott and J. Trotter, *Chem. Comm.* 310 (1965).

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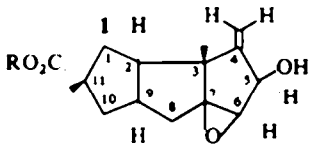
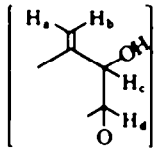
‡ Herein referred to simply as hirsutic acid.

[1] N. G. Heatley, M. A. Jennings and H. W. Florey, Brit. *J. Exp. Pathology* **28**, 35 (1947).

[2] A. I. Scott, *Interpretation of the Ultraviolet Spectra of Natural Products*. Pergamon, Oxford (1964).



I



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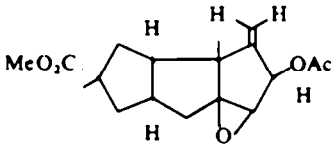
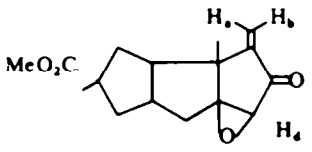


III



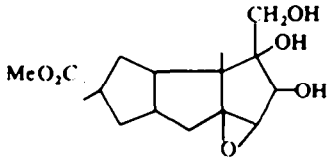
IV

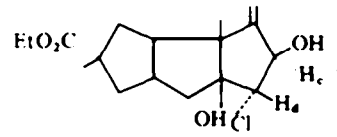
double bond was shown by the formation of methyl hirsutate monoacetate (III) τ 7·85 (acetate C$\underline{H}_3$) in which the tertiary proton H_c underwent a characteristic NMR shift [τ 5·40 (C$\underline{H}$OH) → τ 4·98 (C$\underline{H}$OAc)] with concomitant disappearance of the OH band at 3500 cm^{-1} in the IR. Furthermore, manganese dioxide oxidation of methyl hirsutate afforded an $\alpha\beta$-unsaturated ketone (IV) λ_{max} 231 (ε 5350) ν 1727 cm^{-1} indicative of an exomethylene cyclopentanone (λ calc.[2] 230 mμ) H_a, H_b, H_d now appeared as singlets in the NMR indicating the loss of H_c and confirming the long range coupling assignments.

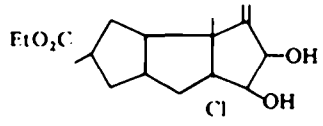
Chemical confirmation of the exocyclic double bond was obtained in several ways. Thus ozonolysis of methyl hirsutate gave formaldehyde (as the 2,4-dinitrophenylhydrazone) and catalytic reduction (Pt) of the ester afforded dihydromethyl hirsutate whose NMR spectrum was consistent with the replacement of exomethylene by secondary methyl (τ 9·05; 3 proton doublet; J = 6·0 c/s). Finally osmium tetroxide oxidation of the ester afforded a triol (V).

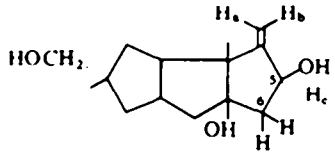
The presence of the last remaining oxygen function as epoxide adjacent to the secondary OH group was suggested by formation of a chlorohydrin (VI) by treatment of hirsutic acid with ethanolic hydrogen chloride.

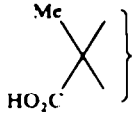
The IR spectrum of VI showed enhanced OH absorption at 3500 cm^{-1} as well as peaks at 1720 and 1190 cm^{-1} (ethyl ester). In addition the NMR spectrum of VI showed ethyl ester (τ 5·86 q, 8·76 t, J = 7 c/s) H_d now appeared at τ 6·04 and H_c and H_d displayed increased coupling (J H_c, H_d = 9 c/s). The assignment VI rather than the alternative VII was indicated by (i) the failure of VI to form an isopropylidene derivative, (ii) a negative reaction with periodate and, (iii) the formation of a *mono* acetate. The latter showed IR absorption at 3480 (OH) and 1735, 1370 (OAc) cm^{-1}. The epoxide function of I (R = Me) was reduced with LAH to the *triol* VIII which displayed OH at 3500 cm^{-1} but no carbonyl absorption. The mass spectrum of VIII had, in addition to a molecular ion (M^+; *m/e* 252), peaks due to loss of one and two hydroxyls and hydroxymethyl functions at *m/e* 234 (M-18), 216 (M-36) and 221 (M-31; CH_2OH). The CH_2OH function was evident as a two proton singlet in the NMR spectrum at τ 6·70. Both tertiary methyls appeared in the triol VIII at τ 8·90 (resolved at 100 mc). This fact suggested the grouping IX in the acid. The rather complex pattern of H_a, H_b and H_c in VIII was interpreted as follows. The exomethylene

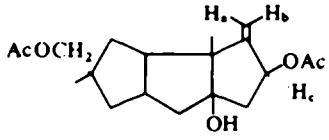

V


VI


VII

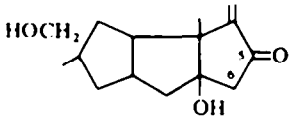

VIII

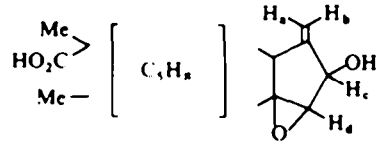

IX

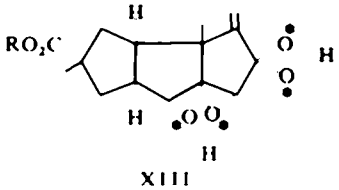

X

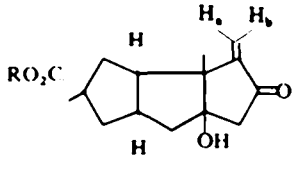
protons H_a and H_b appeared in quartets (τ 4·82, 5·03) with long range couplings H_a, H_c (1·8 c/s) and H_b, H_c (1·9 c/s). H_a and H_b showed a geminal splitting of 0·15 c/s. The C_5 (H_c) proton was a broad multiplet which collapsed on addition of acid in CD_3COCD_3 to a triplet of triplets (pattern 121, 242, 121) $H_{a,b}$, H_c 1·8 c/s; H_c, H_b 6 c/s. At the same time the OH protons at τ 6·19, 6·53 and 7·15 disappeared on acid treatment.

Acetylation of VIII produced a diacetate X (τ 7·94; 6H; $OCOCH_3$). The CH_2OAc protons appeared as a singlet at τ 6·18 with no change in the NMR characteristic of the tertiary Me and exomethylene protons compared with the corresponding data for VIII. H_c however now formed a decet as a triplet, quartet, triplet (121, 1331, 121) ascribed to splittings of 5·80 and 7·0 c/s with the C_6 protons as well as long range splittings (1·9 c/s) with H_a, H_b.


XI


XII


XIII


XIV; R = *p*-bromophenacyl
XV; R = Me

The triol VIII absorbed one molecule of hydrogen and could be oxidized to an αβ-unsaturated ketone (XI) with chromium trioxide pyridine. The latter had λ_{max} 234 mμ and ν 1720 cm^{-1} (exomethylene cyclopentanone). The C_6 protons of XI appeared at τ 7·48.

The structural information provided by these chemical transformations was then summarized as in XII, which accounts for all of the functionality but for only 10 of the 15 carbon atoms, giving little information of the skeleton of the acid and little more than a promising indication of its terpenoid nature.

In view of the scarcity of the acid* and the uncertainty of its future reappearance from natural sources, several rather uninformative dehydrogenation experiments were discontinued and a heavy atom derivative prepared for X-ray diffraction studies. Our choice for this derivative, namely the *p*-bromophenacyl ester (I; R = *p*-bromophenacyl) in the event provided opportunity not only for the completion of the structural work but for a further chemical study. The ester had λ_{max} 255 mμ (ε 15,500) IR 1735 (ester 1705 $\diagdown$C=O) cm^{-1}. Crystallization from benzene afforded the sample used for diffraction as colourless prisms belonging to the space group $P2_12_12_1$.† Initially a set of intensities was collected on film and estimated visually. On the first electron density distribution, calculated using the bromine atom coordinates, 28 of the 29 atoms (excluding hydrogen) were resolved. In subsequent rounds of the analysis the missing atom (epoxide oxygen) was located. However refinement of the positional and isotropic thermal parameters did not proceed as expected and the completed analysis in fact gave positions for 27 full atoms and 4 "half-atoms" as shown in XIII where half-atoms (oxygen) are denoted by asterisks. This suggested that the X-irradiated crystal *now contained an almost equal mixture of two randomly distributed but chemically distinct molecules.* It therefore appeared that a solid phase transformation had occurred without disruption of the crystal structure and with only slight change in the position parameters of two oxygen functions.

It could be shown that this unexpected rearrangement had indeed occurred by preparative X-irradiation of a sample of *p*-bromophenacyl hirsutate. The resultant mixture was separated into unchanged ester (40%) and a new ester to which structure XIV was assigned by combination of X-ray data (summarized in formula XIII and discussed elsewhere)[3] and the following spectroscopic evidence. Thus it can be seen from inspection of XIV that the new ester differs from the bromophenacyl hirsutate at C_5, C_6 and C_7. Bond angle and oxygen positions suggested that a carbonyl group had replaced the secondary OH function and that the epoxide had been opened at C_6 to give a tertiary OH function. In the IR spectrum of XIV the presence of OH was clearly shown (3480 cm^{-1}) whilst the αβ-unsaturated ketone system was characterized by the IR spectrum (ν 1720, 1625 cm^{-1}). In addition the UV spectrum of XIV showed enhanced absorption, λ_{max} 255 mμ (ε 18,500) compatible with an enone chromophore masked by the aromatic absorption band. In the NMR spectrum of XIV the C_6 methylene group appeared as a 2 proton singlet at τ 7·48 whilst the exocyclic methylene hydrogens H_a, H_b no longer display long range coupling with H_c and as in the ketone IV appear as singlets at τ 4·72 and 3·96.

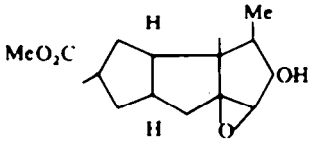
* At this stage some 400 mg remained from the original gift of 3·5 g.

† The details of the X-ray analysis have been published elsewhere[3] but certain facets of this study are pertinent to the chemical investigation and are reported herein.

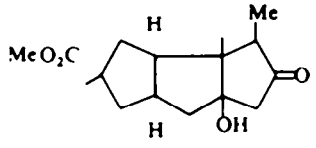
[3] F. W. Comer and J. Trotter, *J. Chem. Soc* B, **11** (1966).

The generality of the X-ray induced rearrangement was now investigated. Solid state irradiation of methyl hirsutate afforded an αβ-unsaturated ketone (λ_{max} 238 mμ) in ca. 30% yield. This proved to be a rather unstable preparation and attempts to purify the ketone gave mixtures of products including (on the basis of UV data) (XV) λ_{max} 246 mμ.

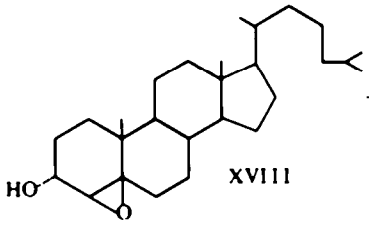
Similarly the dihydro ester XVI afforded an oily transformation product λ_{max} 288 mμ (ε 25), 3450, 1730, 1720, 915 cm^{-1}, to which we ascribe structure XVII.



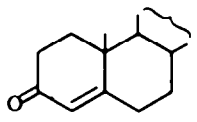
XVI



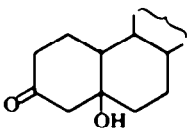
XVII



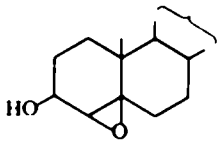
XVIII



XIX



XX



XXI

On the other hand irradiation of hirsutic acid itself in which carboxyl group hydrogen bonds are expected to dominate the molecular packing does not undergo any such rearrangement. Other ring systems bearing α-epoxy OH functionality were examined but little or no rearrangement could be detected in the solid state irradiation of either 4β, 5β-epoxy-cholestan-3α-ol* XVIII or the epimeric 3β-hydroxy compound XXI. Thus the X-irradiation may well be a unique attribute of the crystal structure of the hirsutic ester series.† Similar rearrangements may of course take place in many cases under the influence of X-rays—but the present examples may be unique in that little change in the crystal packing of the isomer is observed, e.g. the bromo ester XIV had the following dimensions before and after irradiation: a = 6·49, 6·56; b = 9·14, 9·38; c = 35·64, 35·14 Å; U = 2114, 2162 Å respectively.

In case this type of rearrangement is in fact more common than hitherto suspected it is suggested that all X-ray diffraction studies should include thin layer chromatographic analysis of the crystal both before and after bathing in X-radiation.

* About 1% of material separated by TLC was tentatively identified (TLC; λ_{max} 240 mμ) as cholest-4-en-3-one (XIX) and about 0·1% as the desired ketol (XX).

† The possibility that the transformation could be thermally induced was excluded by appropriate control experiments.

The absolute configuration of the ester I (R = *p*-bromophenacyl) having been determined by the anomalous dispersion method,[3] it remains to comment on a possible mode of biogenesis for hirsutic acid C. The close familial resemblance with humulene, illudin S[4] and marasmic acid[5] suggests two possible biogenetic schemes as indicated below.

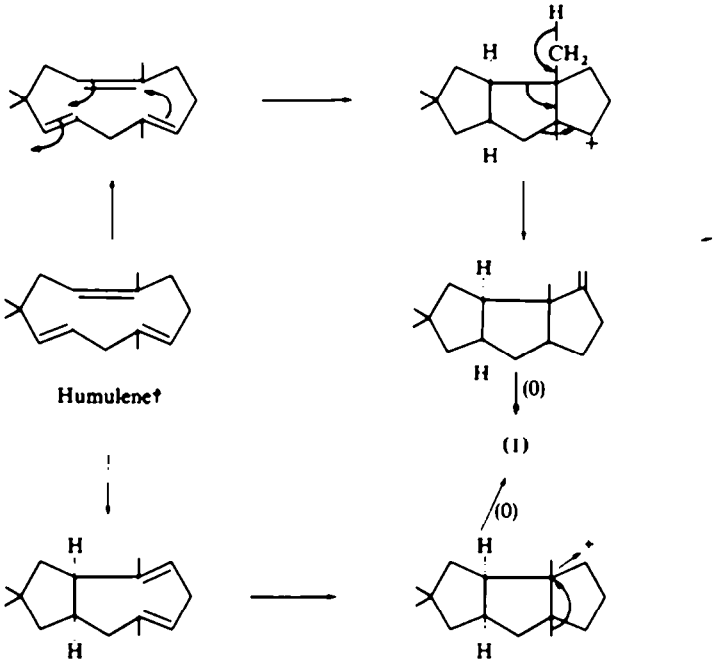


FIG 1.

EXPERIMENTAL

M.ps were determined on the Kofler hot stage microscope. UV spectra in EtOH (Cary 14), IR spectra in $CHCl_3$ (0·1 mm cells; Perkin Elmer 137B) and rotations in $CHCl_3$. NMR spectra were measured in $CDCl_3$ (Varian A-60) and mass spectra on the AEI MS 9 double focussing spectrometer at 70 eV using the direct probe insert method.

X-irradiation experiments were carried out using unfiltered molybdenum radiation, the sample being contained in a thin walled soft glass capsule which was then located in the cavity of a stainless steel block attached to the X-ray tube outlet.

For TLC silica gel G (Stahl) was used and detection carried out by UV irradiation, I_2 vapour or a 5% ceric sulphate–H_2SO_4 spray.

Hirsutic acid (I; R = H). A sample of the crude acid[1] supplied by Dr. N. G. Heatley was recrystallized from EtOH to give colourless prisms m.p. 179–180° $[\alpha]_D^{23}$ + 116° (c, 1·05). *IR*: ν 3520, 3200, 2950, 1700, 1660, 920, 890 cm^{-1}; *UV*: End absorption; NMR*: τ 4·73 d, 5·00 d (H_a, H_b = 1·9, 2·6 *J* H_a, H_b = 0 c/s) 5·39 m

* s = singlet; d = doublet; q = quartet; m = multiplet

† Natural humulene is of course the all-*trans* triene.[6] We have shown a *cis,trans,trans*-isomer simply to formalise the spatial correlation of humulene and hirsutic acid.

[4] T. C. McMorris and M. Anchel, *J. Am. Chem. Soc.* **85**, 831 (1963); *Ibid.* **87**, 1594 (1965); M. Tada, Y. Yamada, N. S. Bhacca, K. Nakanishi and M. Ohashi, *Chem. Pharm. Bull., Tokyo*, **7**, 853 (1964); K. Nakanishi, M. Tada and Y. Yamada, *Ibid.* **7**, 856 (1964).

[5] J. J. Dugan, P. de Mayo, M. Nisbet and M. Anchel, *J. Am. Chem. Soc.* **87**, 2768 (1965).

[6] A. T. McPhail, R. I. Reed and G. A. Sim, *Chem. & Ind.* 976 (1964); J. A. Hartsuch and I. C. Paul, *Ibid.* 977 (1964).

(H_c), 6·54 d (H_a, J H_c, H_d = 2 c/s) 8·62 s (C_{11} Me), 8·97 s (C_3—Me), 7·4/8·2/9·0 (8 skeletal protons). (Found: C, 68·30; H, 7·47. $C_{15}H_{20}O_4$ requires C, 68·16; H, 7·65%)

Methyl hirsutate (I; R = Me). Hirsutic acid (1·0 g) was dissolved in ether (30 ml) and the soln cooled to 0°. Ethereal diazomethane (excess) was added so that the temp remained at 0°. Removal of the solvent afforded a quantitative yield of the methyl ester, colourless prisms m.p. 161–162° $[\alpha]_D^{20}$ +119° (*c*, 2·25) IR: 3660, 3500, 1720, 1655, 1160, 915, 885 cm^{-1}. NMR (100 mc): τ 4·72 d, 5·00 d (H_a, H_b; J H_a, H_b = 0; J H_a, H_b: H_c 2·1, 2·7 c/s) 5·40 m (H_c) 6·55 d H_d, J H_c, H_d = 1·9 c/s), 6·34 s (OCH_3), 8·66 s (C_{11}—CH_3), 8·97 s (C_3 Me), 7·4/8·2/9·0 (8 skeletal protons). *Mass spectrum*: *m/e* 278 (M^+ 263 (M—CH_3) 260 (M—H_2O), 219 (M CO_2CH_3) 201 (219$^+$ H_2O). (Found: C, 69·09; H, 7·98. $C_{16}H_{22}O_4$ requires: C, 69·04, H, 7·97%)

Triol (VIII). A soln of methyl hirsutate (500 mg) in THF (25 ml) was added dropwise to a refluxing soln of LAH (2 g) in THF (100 ml) and the mixture reluxed for 5 hr. Decomposition of excess LAH with AcOEt and work up in the usual way gave colourless prisms of VIII (30%) m.p. 117 118° $[\alpha]_D^{19}$ +54 (*c*, 2·10). IR: ν 3500, 2900, 1655, 910 cm^{-1}; NMR (100 mc): τ 4·82 q, 5·03 q (H_a, H_b; J H_a, H_b = 0·5; J H_a, H_b: H_c = 1·8, 1·9), 5·50 (H_c), 6·70 s (CH_2OH), 8·90 d (6H C_3/C_{11} Me; J = 1 c/s) 7·4/9·0 (8 skeletal protons). In $(CD_3)_2CO$: τ 4·91, 5·10, 5·6, 6·76, 8·92, 7·4 9·0 and peaks at 6·19, 6·53, 7·15 (OH) which disappear on addition of HCl. *Mass spectrum*: *m/e* 252 (M^+); 234 (H_2O) 221 (CH_2OH) 216 ($-2H_2O$) 31 (CH_2OH^+). (Found: C, 70·92; H, 9·09. $C_{15}H_{24}O_3$ requires: C, 71·39; H, 9·59%)

Methyl hirsutate acetate (III). Methyl hirsutate (145 mg) was dissolved in dry pyridine (2 ml) and Ac_2O (2 ml) added. After a few days removal of solvents at 10^{-4} mm gave a colourless oil which distilled at 85°/0·02 mm $[\alpha]_D^{23}$ +106° (*c*, 1·78) IR: ν 2990, 1735, 1725, 1675, 1375, 1210, 1170, 915, 875; NMR: τ 4·29 d, 4·92 d (H_a, H_b; J H_a, H_b = 0 J H_a, H_b: H_c = 2·2, 2·2) 4·98 m (H_c), 6·44 d (H_d; J H_c, H_d = 2·0), 6·33 s($COOMe$), 7·4/8·2/9·0 (8 Protons). (Found: C, 67·87; H, 7·59. $C_{16}H_{24}O_5$ requires: C, 67·48; H, 7·55%)

Oxidation of methyl hirsutate with MnO_2. Active MnO_2 was added to a soln of the ester (155 mg) in $CHCl_3$ (15 ml) and the mixture shaken for 24 hr. Removal of solvent from the filtered solution and crystallization from benzene pet. ether (b.p. 60–80°) gave colourless prisms of IV (50%) m.p. 103 104° $[\alpha]_D^{22}$ −96° (*c*, 2·23). UV: 231 mμ (ε 5350); IR: ν 2980, 1727, 1720, 1645, 1170, 915, 885; NMR: τ 3·92 s, 4·69 s (H_a, H_b; J H_a, H_b = 0) 6·58 s (H_d), 6·34 s (ester Me), 8·26 s (C_{11} Me), 8·82 s (C_3 Me) 7·3/8·1/9·0 (8H). (Found: C, 69·61; H, 7·34. $C_{16}H_{20}O_4$ requires: C, 69·54; H, 7·30%)

Dihydro methyl hirsutate (XVI). The ester (50 mg) was hydrogenated in AcOEt soln over pre-reduced 5% Pd C (70 mg) until 1 mole H_2 was absorbed (3 min) to give XVI as prisms from AcOEt m.p. 119–120° $[\alpha]_D^{23}$ +25 (*c*, 1·70). IR: ν 3600, 3460, 2980, 1720, 1165, 920, 885 cm^{-1}; NMR: τ 5·9 (H_c), 6·77 d (H_d; J H_c, H_d = 2·0 c/s) 6·40 s (ester Me), 8·70 s (C_{11}—Me), 9·10 s (C_3 Me), 9·05 d (C_4 Me; J = 6·0 c/s). (Found: C, 67·99; H, 8·48. $C_{16}H_{24}O_4$ requires: C, 68·54; H, 8·64%)

The triol V. A soln of methyl hirsutate (200 mg) in dioxan (10 ml) containing OSO_4 (267 mg) was stored in the dark for 14 days. H_2S was passed through the black soln and the osmium sulphide removed by filtration through a celite pad. The triol was recovered as an amorphous *solid*, m.p. 165–173° (10%) $[\alpha]_D^{22}$ 0° (*c*, 1·27). IR: ν 3500, 2970, 1725, 1180, 915, 875; NMR (dimethylformamide): τ 6·35 s (ester Me), 8·61 s (C_{11} Me) 9·08 s (C_3 Me). (Other protons poorly resolved). (Found: C, 60·94; H, 7·44. $C_{16}H_{24}O_6$ requires: C, 61·52; H, 7·75%)

Ozonolysis of methyl hirsutate. Ozonized O_2 was passed through a soln of methyl hirsutate (53 mg) in CH_2Cl_2 at −78° (20 ml) for 1 hr. The ozonide was decomposed by warming with water and the reaction mixture steam distilled. The distillate afforded a 2,4-dinitrophenylhydrazone m.p. 162–163° undepressed on admixture with authentic formaldehyde 2,4-dinitrophenyl hydrazone.

Ethyl hirsutate chlorohydrin (VI). Hirsutic acid (100 mg) was dissolved in EtOH (20 ml) and conc HCl (2 ml) added. The soln was heated under reflux for 5 hr and the product isolated by ether extraction to give prisms of the chlorohydrin (52 mg) m.p. 95 96° $[\alpha]_D^{23}$ +67° (*c*, 2·52) UV: end absorption; IR: ν 3610, 3480, 2980, 1720, 1665, 1190, 910 cm^{-1}. NMR: τ 4·68 d, 4·81 d (H_a, H_b; J H_a, H_b = 0; J H_a, H_b: H_c − 2·0, 2·0 c/s) 5·7 (H_c), 6·04 d (H_d; J H_c, H_d = 9 c/s), 5·86 q, 8·76 t (ester Et, J = 7 c/s), 8·67 s (C_{11}—Me), 8·87 s (C_3 Me) 7·4/8·4/9·0 (8H).

p-Bromophenacyl hirsutate (I; R = *p*-bromophenacyl). Hirsutic acid (150 mg) and *p*-bromophenacyl bromide (160 mg) were dissolved in dry acetone (15 ml) containing K_2CO_3 (1·0 g) and the mixture heated under reflux for 50 min. Filtration, removal of acetone and recrystallization from benzene–EtOH gave prisms of the *p*-bromophenacyl ester (50%) m.p. 129–130° $[\alpha]_D^{22}$ +97° (*c*, 1·55). IR: ν 3600, 3500, 2920, 1735, 1705, 1660, 1590, 920, 890 cm^{-1}; UV: λ_{max} 255 mμ (ε 15000); NMR: τ 2·18, 2·37 (4 Ar-H; J = 9 c/s), 4·71 s (phenacyl CH_2), 4·70 d, 4·98 d (H_a, H_b; J H_a, H_b = 0; J H_a, H_b: H_c = 2·1) 6·36 (H_c) 6·52 d (H_d; J H_c, H_d = 2·1 c/s), 8·57 s (C_{11}—Me), 8·95 s (C_3—Me) 7·3/8·2/9·0 (8H); *Mass spectrum*: *m/e* 460, 462 (M^+)

445, 447 (—Me), 442, 444 (· H_2O) 263 (—$CH_2COC_6H_4Br$), 245 (263 H_2O) 219 (—$CO_2CH_2COC_6H_4Br$) 201 (209* ··H_2O). (Found: C, 60·08; H, 5·78; Br, 17·44. $C_{23}H_{25}O_5Br$ requires: C, 59·88; H, 5·46; Br, 17·30%.)

X-irradiation experiments

(i) *p-bromophenacyl hirsutate* (I; R = *p*-bromophenacyl (200 mg) was exposed to Mo radiation for 60 hr. Preparative TLC using two silica gel plates (20 × 60 × 0·05 cm) containing G.E. phosphor and developed along the axis with benzene:ether 1:4 for 24 hr. The unchanged ester (60% on this scale; 40% using 3 mg) was recovered with ether. The *iso* ester (XIV) 40% on this scale; 60% using 3 mg) did not crystallize. The oil showed single spot TLC characteristics (R_f 0·45 in benzene ether (1:4) and had $[\alpha]_D^{19}$ +30° (*c*, 1·10). IR: ν 3480, 2920, 1735, 1720, 1705, 1625, 1585, 910 cm^{-1}; UV: λ_{max} 255 mμ (ε 18,500); NMR: τ 2·20, 2·40 (4ArH), 4·72 (phenacyl —CH_2), 3·96 s, 4·72 s (H_a, H_b; J H_a, H_b = 0), 7·48 s (C_6 CH_2), 8·58 s (C_3—Me), 7·3/8·3/9·0 (8H).

(ii) *Methyl hirsutate*. Bombardment of I (R = Me) with Mo radiation for 150 hr gave XV (30 mg) separated as in (i), IR: ν 3400, 1725, 1620, 1160, 880 cm^{-1}; NMR: τ 4·12 s, 4·82 s (H_a, H_b; J H_a, H_b = 0 c/s) 6·35 s (ester—Me) 8·82 s (C_3—CH_3) 7·2/8·4/9·0 (8H). The rapid deterioration of this compound on storage was accompanied by a change in UV absorption λ_{max} 238 mμ → λ_{max} 246 mμ (ε 11,000).

(iii) 4β,5β-*epoxy*-5β-*cholestan*-3α-*ol*.[7] Irradiation of XIX (30 mg); m.p. 163 164° $[\alpha]_D$ +25° for 7 days gave material m.p. 140 157° λ_{max} 240 mμ (ε 236). TLC showed that the mixture contained starting material 90–95% and at least four new products. TLC comparison showed that two of these were cholest-4-en-3-one λ_{max} 240 (ε 16,000) (1·5%) and XX (0·1%).[8] Similar, prolonged irradiation of hirsutic acid (I; R = H) and of XXI[9] left the crystalline material unchanged in each case.

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[7] Pl. Plattner, H. Heusser and A. B. Kulkarni, *Helv. Chim. Acta* 32, 365 (1949).

[8] Pl. Plattner, H. Heusser and A. B. Kulkarni, *Helv. Chim. Acta* 31, 1885 (1948); E. M. Burgess, *J. Org. Chem.* 27 1433 (1962).

[9] D. J. Collins, *J. Chem. Soc.* 3925 (1959) and Refs. cited.