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THE STRUCTURE OF SULPHURENIC ACID

A NEW TRITERPENOID FROM *POLYPORUS SULPHUREUS*

JOSEF FRIED,[1] P. GRABOWICH, E. F. SABO and A. I. COHEN
Squibb Institute for Medical Research, New Brunswick, N.J.

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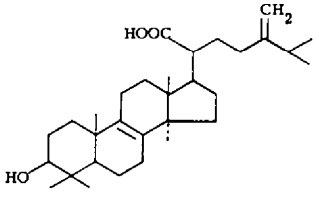
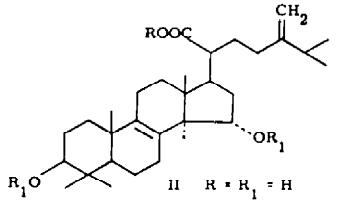
Abstract—A new tetracyclic triterpenoid acid, sulphurenic acid, has been isolated from the mycelium of the fungus *Polyporus sulphureus* grown in both surface and submerged culture, and its structure determined as 15α-hydroxyeburicoic acid (II). It represents 25% of the total steroid content of *P. sulphureus*, the remaind erbeing eburicoic acid. Ergosterol has been isolated from the lipid fraction of this fungus.

OUR interest in the triterpenoid acids of fungal origin as precursors for the synthesis of substances related to the steroid hormones[2] has centred mainly around eburicoic acid (I), which is produced in great abundance by a variety of wood rotting fungi mainly of the genus *Polyporus*.[3] The species *P. sulphureus* was selected because of the ease with which the acid could be isolated from the mycelial growth obtained by both surface[4] and submerged[5] culture of this organism.

It was observed in the course of our work that paper chromatograms of the crude crystalline acids using the system developed by Pan *et al.*[6] always showed, in addition



I



II R = R_1 = H
IIa R = CH_3, R_1 = H
IIb R = CH_3, R_1 = Ac
IIc R = H , R_1 = Ac

to the spot for eburicoic acid, a more polar spot, which became more intense in the mother liquors of the eburicoic acid crystallization. Methylation of such mother liquor fractions with diazomethane gave on preparative thin layer chromatography

[1] Present address: The Ben May Laboratory for Cancer Research, The University of Chicago, Chicago 37, Illinois.

[2] D. Rosenthal, J. Fried, P. Grabowich and E. F. Sabo, *J. Amer. Chem. Soc.* **84**, 877 (1962). [b] G. W. Krakower, J. W. Brown and J. Fried, *J. Org. Chem.* **27**, 4710 (1962). [c] J. Fried and E. F. Sabo, *J. Amer. Chem. Soc.* **84**, 4356 (1962). [d] D. Rosenthal, P. Grabowich, E. F. Sabo and J. Fried, *ibid*, **85**, 3971 (1963). [e] A. I. Laskin, P. Grabowich, C. de Lisle Meyers and J. Fried, *J. Med. Chem.* **7**, (1964).

[3] For comprehensive reviews on tetracyclic triterpenoid acids see: Sir J. Simonsen and H. C. J. Ross in *The Terpenes* Vol. 5; p. 1ff. The University Press, Cambridge (1957), and G. Ourisson and P. Crabbé, *Les Triterpènes Tétracycliques*. Hermann, Paris (1961).

[4] R. M. Gascoigne, J. S. E. Holker, B. T. Ralph and A. Robertson, *J. Chem. Soc.* 2346 (1951).

[5] S. C. Pan and W. R. Frazier, *Biotechnol. and Bioeng.* **4**, 303 (1962).

[6] S. C. Pan, A. I. Laskin and P. Principe, *J. Chromatog.* **8**, 32 (1962).

in addition to the zone for methyl eburicoate a second slower-moving zone, from which the methyl ester of a new acid could be isolated in crystalline form. Because of its derivation from *P. sulphureus* the new acid has been named sulphurenic acid. A quantitative estimation of the acids derived from the mycelium of this fungus grown either in surface of submerged culture indicated a total content of triterpenoid acids of 30%, three fourths of which was accounted for as eburicoic acid and the remainder as sulphurenic acid. No significant amounts of other acidic products were in evidence by either paper or thin layer chromatography. The ubiquitous ergosterol could, however, be isolated in crystalline form from the lipid fraction.[7] An efficient separation of the two acids was achieved by crystallization of their methyl esters from methanol, from which methyl sulphurenate crystallizes in almost quantitative yield. Acetylation of the mother liquor material then furnished pure methyl acetyl eburicoate.

The methyl ester of sulphurenic acid gave correct analytical figures for the formula $C_{32}H_{52}O_4$, which is the composition of methyl eburicoate plus one extra oxygen atom. Like methyl eburicoate, methyl sulphurenate (IIa) possesses a readily reducible double bond terminating in a methylene group as evidenced by an IR band at 11·32 μ and NMR signals at 5·26 and 5·33τ. These spectral features are absent in methyl dihydrosulphurenate (III). The carbomethoxy group of methyl sulphurenate resisted hydrolysis by strong bases at elevated temperatures, which is a characteristic property of all fungal acids bearing carboxyl at C_{21}. Demethylation to the parent acid (II) (m.p. 252–254°, $[\alpha]_D^{23}$ +42° (pyridine)) could, however, be achieved by reagents attacking the methyl carbon rather than the hindered 21-carbonyl group. Most successful in this respect was the reductive demethylation by means of lithium and liquid ammonia described by Wenkert and Jackson[8] for strongly hindered tertiary acids. As an alternative the Taschner procedure[9] using lithium iodide in collidine and employed by us on a previous occasion for demethylations in the eburicoic acid series,[2b] could also be applied, although in this case it was necessary to purify the resulting gelatinous acid via the diacetate IIc.

Methyl sulphurenate could be acetylated at room temperature to form a diacetate (IIb)[10] and it was readily oxidized to a dicarbonyl compound (V) by the Jones reagent. Since the NMR spectrum of V showed no signal for aldehydic protons, both carbonyl groups must be ketonic and therefore derived from secondary hydroxyl groups. The presence of the tetrasubstituted "lanosterol" double bond was demonstrated by oxidation of methyl diacetyl dihydrosulphurenate (IIIa) with chromic acid at 70° to a yellow diketone (IV) possessing the characteristic UV maximum for Δ^2-ene-1,4-diones at 270 mμ (ε = 9,700). The hydroxy keto ester VI prepared in excellent yield by selective borohydride reduction of V at room temperature smoothly underwent the retropinacol rearrangement characteristic of 3β-hydroxy-4,4-dimethyl steroids to

[7] Ergosterol has also been isolated from the mycelium of *P. tumulosus* by L. A. Cort, R. M. Gascoigne, J. S. E. Holker, B. T. Ralph, A. Robertson and T. T. H. Simes, *J. Chem. Soc.* 3713 (1954).

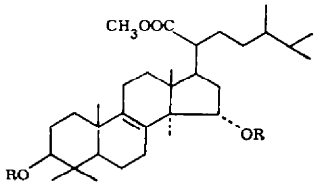
[8] E. Wenkert and B. G. Jackson, *J. Amer. Chem. Soc.* **80**, 217 (1958).

[9] E. Taschner and B. Liberek, *Rocz. Chem.* **30**, 323 (1956).

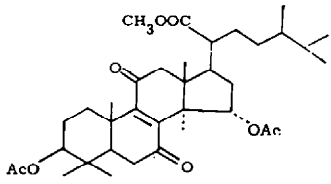
[10] The mass spectrum of methyl diacetyl sulphurenate (direct inlet system) showed the molecular ion peak at 584 and significant peaks at M-60 (CH_3COOH), M-75 ($CH_3COOH + CH_3$) and M-135 ($2CH_3COOH + 15$). Methyl acetyl eburicoate showed $M^+ = 526$, M-15 (CH_3) and M-75 ($CH_3COOH + CH_3$). We wish to thank Professor Carl Djerassi of Stanford University for these measurements.

form the isopropylidene ketone VIII, readily recognized by its NMR signals at 8·24 and 8·38τ.

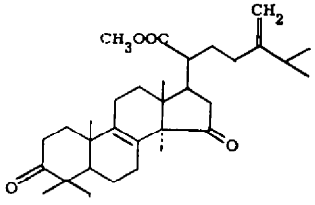
Conclusive evidence for the presence of the eburicoic acid skeleton in sulphurenic acid was obtained by Wolff–Kishner reduction of the acetate of the above described hydroxyketone (VIa) under the rigorous Barton conditions[11] (but not according to Huang-Minlon) to afford after remethylation a 25% yield of methyl eburicoate



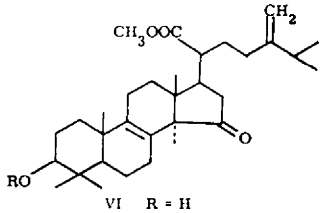
III R = H
IIIa R = Ac



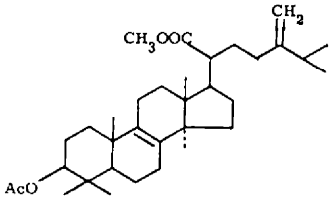
IV



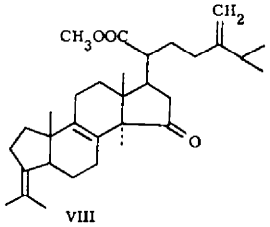
V



VI R = H
VIa R = Ac
VIb R = H, 24(28)-dihydro



VII



VIII

identified as the acetate VII. Thin layer chromatography furnished as the only other crystallizable substance a product of very similar polarity (m.p. 169–171°; $[\alpha]_D$ +26°) in too small an amount for full characterization, in which the 24(28)-methylene group was no longer present. Because of the relatively low yield of methyl acetyl eburicoate in the Wolff-Kishner reduction this latter compound was itself subjected to the conditions of this reaction in order to determine its fate, once formed. Again, the reaction mixture furnished, after methylation, methyl eburicoate and its companion substance in approximately the same yields as found with the keto methyl ester VIa. Sulphurenic acid is therefore a monohydroxy eburicoic acid. Before proceeding to describe the experiments, which unambiguously establish the position of this secondary hydroxyl group we can eliminate certain structural alternatives from further consideration. Thus, the diketone V does not possess the properties of a 1,2- or a 1,3-diketone

[11] D. H. R. Barton, D. A. J. Ives and B. R. Thomas, *J. Chem. Soc.* 2056 (1955).

(negative ferric chloride-test) thereby ruling out positions 1, 2 and 22; nor does it show selective absorption above 200 mμ, which eliminates positions 7, 11 and 23 from further consideration. Moreover, the 16-position is excluded because of the non-identity of sulphurenic acid with tumulosic acid (16α-hydroxyeburicoic acid)[7] and the dextro-rotatory contribution of the keto group under discussion in the sulphurenic acid derivatives V, VI and VIa (Table 2) as contrasted with the powerful levorotatory increment of the 16-keto group.[12] This leaves positions 6, 12 and 15 as possible sites for the second hydroxyl group in sulphurenic acid.

Confirmation of the above considerations and evidence favoring assignment of the hydroxyl group to the 15α-position was obtained from a comparison of the NMR spectra of appropriate derivatives of eburicoic acid and sulphurenic acid. Table 1 shows the important signals and their structural assignments.[13] We note that whereas the spectra of the two series of derivatives are in excellent agreement as far as the signals for the 19, 26, 27, 30 and 31-methyl groups are concerned, the signals for the 18 and 32-methyl groups are shifted to lower field in the sulphurenic acid series. Thus, the 18-methyl group is deshielded to the extent of 0·05 ppm in methyl sulphurenate (IIa) and to the extent of 0·09 ppm in the diacetate IIb and the hydroxyketone VIa as compared with the corresponding 15-unsubstituted derivatives. Kawazoe *et al.*[14] have assessed the extent of deshielding of the 18-methyl group by 15-hydroxyl and acetoxy groups in either configuration. These authors found the effect of the 15β-hydroxyl and acetoxy groups to be 0·27 and 0·23 ppm, respectively, whereas the effect of the corresponding α-oriented substituents was 0·04 and 0·07 ppm, respectively. These latter figures are in good agreement with our values and suggest the presence of a 15α-hydroxyl group in sulphurenic acid. The deshielding of the 32-methyl group by the neighbouring hydroxyl and keto groups to the extent of 0·11 ppm and 0·22 ppm, respectively, supports this assignment rather than the alternative ones involving the 6- and 12-positions.

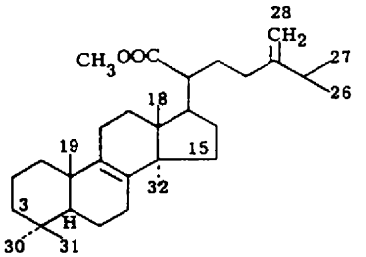
Having thus singled out the 15α-position as the most probable site for the second hydroxyl group in sulphurenic acid, it was our plan to bring about conclusive proof by making use of an intramolecular reaction involving functional groups unique to this formulation. Specifically, this envisioned base-catalyzed cyclization of the 15,24-diketone X to form a pentacyclic indenone derivative (XI), the structure of which, as far as rings D and E are concerned, bears a striking resemblance to the ring C and D portion of the alkaloid jervine (XII) shown here as the d-enantiomorph in a somewhat unorthodox presentation, in order to stress the close similarity in structure between the pertinent portions of these two ring systems. Evidence for the formation of such a compound and comparative data linking the two systems would constitute such proof. To achieve this objective methyl sulphurenate (Ia) was converted by ozonolysis to the 24-keto ester IX and the latter oxidized with Jones reagent

[12] The molecular rotation increments for ten 16-ketosteroids with different side chains range from −470 to −700°. Specifically, the $\Delta[M]_D$ for the 16-keto group in methyl 3,16-diketo-$\Delta^{7,9(11)}$-eburicadiene-21-oate is −450° (A. Bowers, T. G. Halsall, E. R. H. Jones and A. J. Lemin, *J. Chem. Soc.* 2548 (1953).

[13] These assignments were made on the basis of a large number of spectra of eburicoic acid derivatives and their degradation products. A manuscript by A. I. Cohen, D. Rosenthal, G. W. Krakower and J. Fried summarizing this work is in preparation.

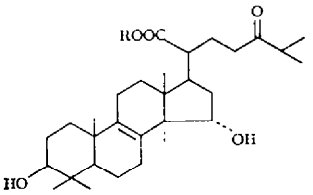
[14] Y. Kawazoe, Y. Sato, M. Natsume, H. Hasagawa, T. Okamoto and K. Tsuda, *Chem. Pharm. Bull., Japan* **10**, 338 (1962).

TABLE 1. NMR SPECTRA OF DERIVATIVES OF SULPHURENIC AND EBURICOIC ACIDS

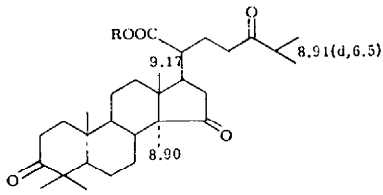


	Structural variant C-3	C-15		Chemical shift, Tau[a] C-3αH	CH_3CO_2-	-OCH_3	C-15βH	28-CH_2	18-CH_3	19-CH_3	30,31-CH_3	32-CH_3	26,27-CH_3
	β-OH	H		6·78	—	6·33	—	5·33(m) 5·26(m)	9·27	9·01	9·20 9·04	9·12	8·99(d, 6)
	β-OH	H	24(28)-dihydro	6·78(m)	—	6·33	—	—	9·27	9·00	9·18 9·03	9·12	9·18(m)[b] 9·12(m)
IIa	β-OH	α-OH		6·75(m)	—	6·33	5·73(m)	5·33(m) 5·26(m)	9·22	9·00	9·22 9·04	9·01	8·98(d)
VIb	β-OH	Oxo	24(28)-dihydro	6·70(m)	—	6·27	—	—	9·18	8·98	9·18 9·01	8·90	9·18(m)[b]
V	Oxo	Oxo		—	—	6·27	—	5·31(m) 5·20(m)	9·15	8·89-8·93	8·89–8·93	8·89–8·93	8·99(d, 6)
VII	β-OAc	H		5·62(m)	7·94	6·33	—	5·31(m) 5·23(m)	9·27	9·00	9·12	9·12	9·00(d, 6)
IIb	β-OAc	α-OAc		5·45(m)	7·95	6·33	4·97(m)	5·37(m) 5·26(m)	9·18	8·99	9·11	8·99	9·01(d)

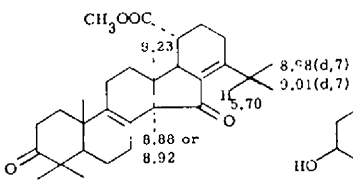
[a] In parentheses: m-multiplet, d-doublet; J in c/s.
[b] C-28 methyl is also included.



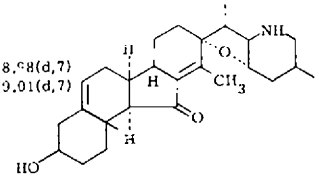
IX R = CH_3
IXa R = H



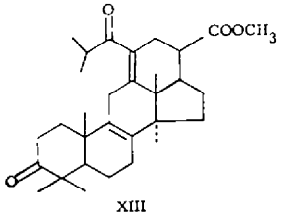
X R = CH_3
Xa R = H



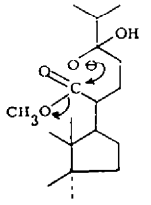
XI



XII



XIII



XIV

to the triketo ester X. Attempts at cyclization in the presence of methanolic KOH did not produce the desired result as shown by the absence of $\alpha\beta$-unsaturated ketone absorption in the ultraviolet. This result was not entirely unforeseen in view of the resistance expected of a *trans*-fused indanone to enolize towards C_{16}. There occurred, however, under these conditions a partial hydrolysis of the carbomethoxy group, which is to be contrasted with the remarkable stability of methyl sulphurenate under the same conditions, and which will be discussed later in this paper. Cyclization was eventually achieved by the use of the stronger base potassium t-butoxide in t-butanol.[15] Under these conditions there was formed the crystalline triketo acid Xa, so identified by reconversion to its methyl ester (X), and an amorphous acid possessing UV absorption at 251 mμ, which on methylation gave rise to the desired pentacyclic ester XI. Its ultraviolet spectrum showed peaks at 251 mμ (ε = 13,200) and 356 mμ (ε = 60) in striking parallel to those of jervine[16] (λ_{max}^{alc} 252 mμ (14,000), 360 mμ (70)), particularly with regard to the low intensity band, which appears at an unusually high wavelength. Equally characteristic was the infrared spectrum of XI, which showed bands of equal intensity at 5·88 μ and 6·18 μ diagnostic of *cisoid* $\alpha\beta$-unsaturated

[15] It was found essential to terminate the reaction after 2 to 3 hr, since longer reaction times gave rise to substances possessing a different chromophore (λ_{max}^{alc} 264 mμ), which could not be crystallized.

[16] W. A. Jacobs and C. F. Huebner, *J. Biol. Chem.* **170**, 635 (1947); J. Fried, O. Wintersteiner, M. Moore, B. M. Iselin and A. Klingsberg, *J. Amer. Chem. Soc.* **73**, 2970 (1951).

ketones,[17] and coinciding with those of jervine (λ_{max}^{Nujol} 5·88 μ, 6·16 μ).[18] These spectral data also serve to distinguish our assigned structure XI from an alternative structure (XIII), which would have been expected had the second hydroxyl group of sulphurenic acid been located at position 12. While it is true that such a chromophore would be predicted to possess UV-absorption similar to that of XI and of jervine (although not with respect to the low intensity band) its infrared spectrum would display bands of unequal amplitude (*transoid* enone) at about 6·00 and 6·15 μ which is, of course, contrary to observation. Moreover, the 8,9-double bond would most likely have entered into conjugation with the $\alpha\beta$-unsaturated ketone system to give rise to a conjugated dienone system.

Confirmation of the assigned structure XI was obtained from an analysis of its NMR spectrum and from a comparison with that of its triketone precursor (X). The pertinent chemical shifts (τ) are indicated in structures X and XI and the full spectrum of XI is shown in the upper portion of Fig. 1. We note that while the chemical shifts for the 32-methyl group are essentially the same in the two compounds, the doublet for the 26 and 27-methyl groups located at 8·91τ in X moves upfield in XI and resolves into two pairs of doublets at 8·98 and 9·01τ. The latter chemical shift is characteristic of compounds possessing a carbon-carbon double bond attached to the isopropyl group (Table 1). A most interesting feature of the spectrum of XI is a multiplet centred at 5·70τ representing a single proton. Only the proton attached to C_{25} could be rationalized to give rise to absorption at such low field, because of its location in the area of maximum deshielding by the 15-keto group. Inspection of Dreiding models shows that free rotation of the isopropyl group about the 24,25-bond is sterically impeded because of potential non-bonded interactions between the 26- and 27-methyl groups and the 15-keto group. This causes the C-25 hydrogen to reside essentially in the plane of the 15-keto group, which is the area of maximum deshielding by that group.[19] The lack of free rotation of the isopropyl group is also considered to be responsible for the difference in chemical shift by 0·03 ppm in the signals of its two methyl groups.

Evidence that the 5·70τ multiplet and the doublets centred at 8·98 and 9·01τ do indeed originate in the isopropyl group and are spin-coupled to each other was obtained by double resonance spectroscopy. The NMDR spectrum of XI is shown in the lower portion of Fig. 1. Sweeping the 5·70τ signal of the highly deshielded proton while irradiating the methyl protons of the isopropyl group at a sideband frequency of 196 c/s, the mean separation in chemical shift between the two signals, shows the former as a singlet. Conversely, sweeping the signals for the methyl groups while irradiating the low field proton (−196 c/s) causes the two pairs of doublets to collapse into two singlets. This is evident from the increase in the height of these peaks in relation to the 18-methyl peak, and their downfield movement by about 4 c/s to the centre of the undecoupled doublets. These findings while again incompatible with structure XIII lend full support to structure XI for the cyclization product, and therefore to attaching the hydroxyl group at position 15. Its α-orientation follows from the NMR data discussed above and summarized in Table 1,

[17] R. L. Erskine and E. S. Waight, *J. Chem. Soc.* 3425 (1960).

[18] B. M. Iselin and O. Wintersteiner, *J. Amer. Chem. Soc.* **77**, 5318 (1955).

[19] L. M. Jackman, *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry* p. 124. Pergamon Press, New York (1959).

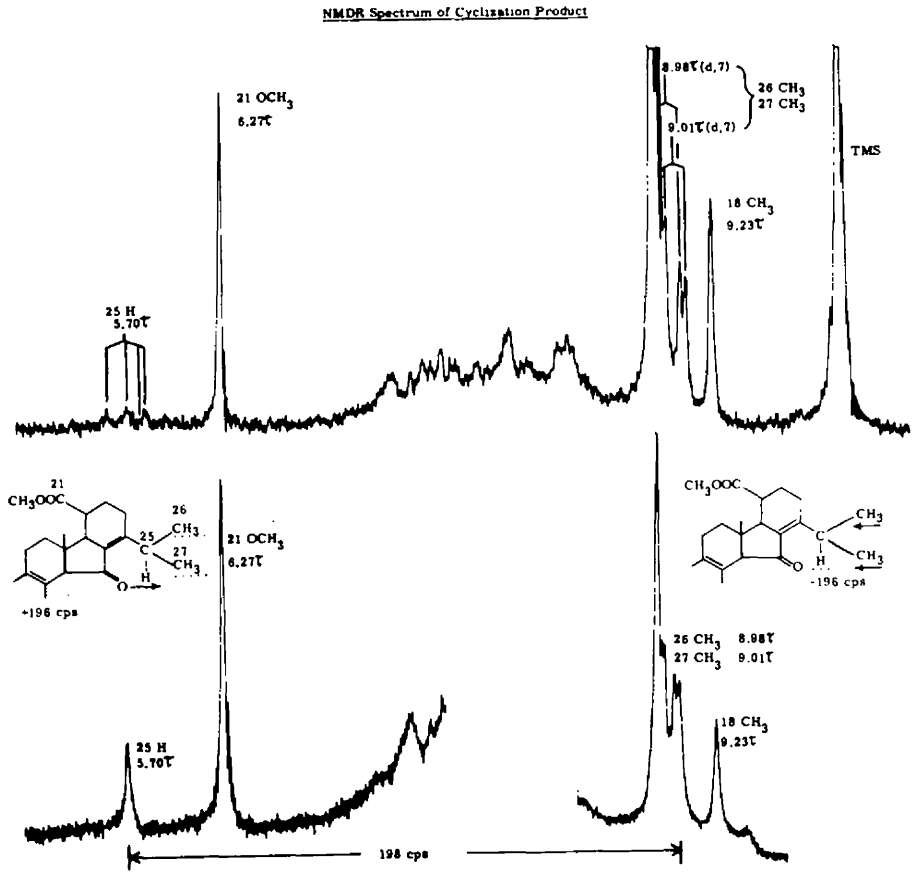
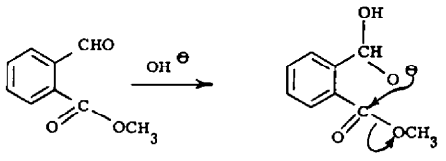


FIG. 1. Top: NMR spectrum of XI in $CDCl_3$ on Varian HR-60 instrument. Lower left: Same as above but 26 and 27-methyl protons are decoupled. Lower right: Same as above but C-25 proton is decoupled. Direction of arrow indicates which proton(s) was spin-decoupled. . . . shows the proton that was irradiated with the side band frequency.

from its ready acetylability[20] and from a comparison of the positive molecular rotation increments for both the hydroxyl and acetoxy groups shown in Table 2 with those of a wide variety of known 15α- and β-hydroxy and acetoxy steroids (Table 3).

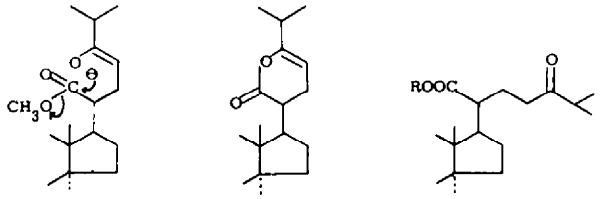
We observed earlier in this paper that the 24-keto ester X was partially hydrolyzed by methanolic KOH, whereas the esters of eburicoic acid are notoriously stable under much more rigorous conditions. This observation has been made the basis for an efficient hydrolysis procedure for 24-keto methyl esters. Thus, the ester IX on treatment with boiling N KOH in ethanol-water for 5 hr gave the acid IXa in 70% yield. This increased proclivity to hydrolysis is most satisfactorily accounted for by primary attack of hydroxyl ion on the 24-keto group, to form the anion XIV, which, in turn attacks the carbomethoxy group. A similar intermediate (XVI) has

[20] 15β-Hydroxyprogesterone is only partially acetylated with acetic anhydride and pyridine at room temp.



XV XVI

been postulated in the alkaline hydrolysis of methyl *o*-formylbenzoate, (XV) which proceeds at a rate 10^5 times faster than that of methyl benzoate.[21] An alternative mechanism is possible in our case (but not with XV), namely, attack on the carbomethoxy group by the enolate anion XVII to form as an intermediate the enol lactone XVIII, which in turn is hydrolyzed to the parent acid. That this latter pathway is not



XVII XVIII XIX R = H
XIXa R = CH_3
XIXb R = C_2H_5

TABLE 2. MOLECULAR ROTATION CONTRIBUTIONS OF 15-SUBSTITUENTS IN SULPHURENIC ACID DERIVATIVES

Compound	$[\alpha]_D$[a]	$[M]_D$	$\Delta[M]_{15\alpha OH}$[b]	$\Delta[M]_{15\alpha OA_c}$[b]	$\Delta[M]_{15\text{-Keto}}$[b]
Eburicoic Acid (I)[c]	+39(Py)	+184			
Sulphurenic Acid (II)	+42(Py)	+210	+26(Py)		
Acetyl Eburicoic Acid[c]	+42	+215			
Diacetyl Sulphurenic Acid (IIc)	+58	+33		+116	
Methyl Eburicoate[c]	+45	+218			
Methyl Sulphurenate (IIa)	+66	+330	+112		
Methyl 15-Ketoeburicoate (VI)	+71	+354			+136
Methyl Acetyl Eburicoate (VII)[c]	+49	+258			
Methyl Diacetyl Sulphurenate (IIb)	+67	+391		+133	
Methyl Acetyl 15-Ketoeburicoate (VIa)	+67	+362			+104
Methyl Acetyl 7,11-Diketodihydro-eburicoate[d]	+81	+451			
Methyl Diacetyl 7,11-Diketodi-hydrosulphurenate (IV)	+79	+486		+35	
Methyl 3-Ketoeburica-8,24(28)-diene-21-oate[e]	+59·5	+287			
Methyl 3,15-Diketoeburica-8,24(28)-diene-21-oate (V)	+84	+418			+131

[a] Rotations in $CHCl_3$ unless otherwise indicated.
[b] Signifies the contributions to the molecular rotation by the group shown.
[c] R. M. Gascoigne, J. S. E. Holker, B. J. Ralph and A. Robertson, *J. Chem. Soc.* 2346 (1951).
[d] F. N. Lahey and P. H. A. Strasser, *J. Chem. Soc.* 873 (1953).
[e] R. M. Gascoigne, A Robertson and J. J. H. Simes, *J. Chem. Soc.* 1830 (1953).

[21] M. L. Bender and M. S. Silver, *J. Amer. Chem. Soc.* **84**, 4589 (1962).

TABLE 3. MOLECULAR ROTATION CONTRIBUTIONS OF 15-SUBSTITUENTS IN STEROIDS OF KNOWN STRUCTURE

Parent substance	$\Delta[M]_D$ 15α-OH	15α-OAc	15β-ol	15β-OAc	15-Keto
Progesterone	+120[a]	−31[i]	−105[a]		+53[a]
Cortexone[j]	+92(EtOH)[b]	−1(EtOH)[b]	−123[b]		
Cortexolone[k]	+110(MeOH)[c]	+36[c]	−158[c]	−67[c]	+23[c]
			−70(MeOH)[g]		
Androstenedione	+107(MeOH)[c]		−116[c]		
Estrone[l]	+141(Diox)[d]	+314[d]	−177[e]		
Estradiol[l]	+249(Diox)[d]	+370[d]	−123[e]		
Methyl 3-Keto-5β-androstane-17β-carboxylate	+98[f]		−104[f]		+43[f]
3β-Acetoxyergostane					+106[g]
22a,25a,5α-Spirostane-2α,3β-ol			−91[h]		−35[h]

[a] J. Fried, R. W. Thoma, D. Perlman, J. E. Herz and A. Borman, *Recent Progress in Hormone Research* **11**, 158 (1955); [b] A. Wettstein, *Experientia* **11**, 465 (1955), *Helv. Chim. Acta* **38**, 381 (1955); [c] S. Bernstein, M. Heller, L. I. Feldman, W. S. Allen, R. H. Blank and C. E. Linden, *J. Amer. Chem. Soc.* **82**, 3685 (1960); [d] A. I. Laskin, P. Grabowich, B. Junta, C. de Lisle Meyers and J. Fried, *J. Org. Chem.* **29**, (1964); [e] E. W. Cantrall, R. Littell and S. Bernstein, *J. Org. Chem.* **29**, 64 (1964); [f] A Lardon, H. P. Sigg and T. Reichstein, *Helv. Chim. Acta* **42**, 1457 (1959); [g] D. H. R. Barton and G. F. Laws, *J. Chem. Soc.* 52, (1954); [h] C. Djerassi, T. T. Grossnickle and L. B. High, *J. Amer. Chem. Soc.* **78**, 3166 (1956); [i] J. Fried, R. W. Thoma, D. Perlman and R. W. Gerke, U.S. Patent 2,879,280. [j] Parent substance for the calculation of the 15α-OAc value is cortexone acetate. [k] Parent substance for the 15α-OAc, 15β-OAc values and the higher of the 15β-ol values is cortexolone acetate. [l] Parent substances for the 15α-OAc values are estrone 3-acetate and estradiol 3,17-diacetate, respectively, for the 15β-ol values it is estrone and estradiol 3-methyl ether, respectively.

the correct one was shown by the observation that when the enol lactone XVIII (3β-acetoxy-$\Delta^{8,23}$-lanostadiene-24-ol-21-oic acid 21 → 24-lactone) was subjected to the conditions that will cause complete hydrolysis of the methyl ester IX to the acid IXa there was obtained instead a 1:1 mixture of the 3β-hydroxy-24-keto acid XIX and its ethyl ester XIXb. This result can be rationalized on the basis of the previous findings[2d] that the enol lactone XVIII undergoes methanolysis rather than hydrolysis when exposed to methanolic K_2CO_3 to yield exclusively the methyl ester XIXa. In the reaction with ethanolic KOH the first step would therefore most likely be ethanolysis, to be followed by (carbonyl-assisted) hydrolysis of the intermediate ethyl ester XIXb, the rate of hydrolysis, in this case being lower (and the reaction therefore incomplete) because of the greater +I-effect of the ethyl versus the methyl group.

A similar enhancement in the rate of hydrolysis was observed with the trihydroxy ester XX prepared by borohydride reduction of the keto ester IX. In this case it is the 24-alcoholate anion that is believed to be the nucleophile responsible for the rate enhancement.

EXPERIMENTAL

M.ps were taken on a Thomas-Hoover apparatus and are corrected for stem exposure. Rotations are in $CHCl_3$ unless otherwise specified. UV spectra were determined on a Cary 11, IR spectra on a Perkin-Elmer 21, and NMR spectra on a Varian A-60 spectrometer in $CDCl_3$ solution with tetramethylsilane as internal standard.

Isolation of methyl sulphurenate (IIa) *from the crude triterpene acid fraction from* Polyporus sulphureus

In fractionating the triterpene acids from *P. sulphureus*,[2a] there were obtained from the $CHCl_3$ extract of the mycelium after removal of the bulk of the eburicoic acid by crystallization several lower melting fractions which are rich in sulphurenic acid. This material, m.p. 255–260° (10 g) was suspended in methanol and an ethereal solution of diazomethane was added until methylation was complete. The solution was filtered from a small amount of insoluble residue and the solvents evaporated *in vacuo*. The residual gum was taken up in methanol–chloroform which resulted in the crystallization of 4·8 g of practically pure IIa, m.p. 190–192°. Recrystallization from acetone gave the pure compound without change in m.p.; $[\alpha]_D^{23}$ +66° (*c*, 0·42); λ_{max}^{KBr} 2·83, 3·01, 5·84, 6·08 and 11·27 μ. (Found (after drying at 140° for 4 hr): C, 76·61; H, 10·67; OMe, 5·73. $C_{32}H_{52}O_4$ (500·74) requires: C, 76·75; H, 10·47; OMe, 6·02%.).

Acetylation of the dried mother liquor material gave after crystallization from methanol 3·2 g pure methyl acetyl eburicoate m.p. 153–155°.

Isolation of ergosterol from the hexane extract of the mycelium of P. sulphureus

Concentration of the hexane extract of the mycelium of *P. sulphureus*[2a] gave a heavy syrup which contained residual anti-foam materials from the tank fermentation. A 100 ml aliquot was chromatographed on 200 g neutral alumina. Elution with 1 l. benzene removed 50 g oil and subsequent elution with 1 l. $CHCl_3$ furnished 10 g material, which crystallized spontaneously from hexane to yield 400 mg ergosterol m.p. 160–162°; $[\alpha]_D$ −117°; rep.[22] m.p. 165°, $[\alpha]_D$ −125°; λ_{max}^{alc} 263, 271, 282 and 294 mμ; NMR 4 vinyl protons and 6 methyl groups. (Found: C, 84·79; H, 11·15. Calcd. for $C_{28}H_{44}O$: C, 84·78; H, 11·18%.). Its acetate, m.p. 170–172°; $[\alpha]_D$ −64°; rep.[22] 179; $[\alpha]_D$ −90°. (Found: C, 81·88; H, 10·79. Calcd. for $C_{30}H_{46}O_2$: C, 82·13; H, 10·57%) had an IR spectrum identical with that of an authentic sample of ergosterol acetate.

Quantitative determination of the triterpenoid acid content of the mycelium of P. sulphureus

The dried mycelium of *P. sulphureus* (11·8 g) grown in submerged culture was sequentially extracted in a Soxhlet apparatus with hexane, chloroform and methanol. From the hexane extract, upon concentration, deposited 143 mg of mixed eburicoic and sulphurenic acids, m.p. 280–290°. From the dried mother liquor (433 mg) ergosterol was obtained by trituration with methanol followed by crystallization from hexane. The chloroform extract (3·26 g) was analyzed as follows: A 100 mg sample of the crystalline residue was methylated in methanol suspension with ethereal diazomethane and the resulting methyl esters separated by preparative thin layer chromatography on activity V neutral alumina using chloroform–hexane 4:1 as a developer. There was obtained 62 mg pure methyl eburicoate m.p. 125–130° (R_F = 0·57) and 20 mg pure methyl sulphurenate m.p. 190–192° (R_F = 0·23), leaving 7 mg on the origin. Extraction with methanol furnished 1·54 g solids from which 100 mg mixed crystalline acids were obtained by crystallization from methanol. The total triterpene acid content is, therefore, 3·5 g or 29·6% of the mycelial weight. The ratio of eburicoic and sulphurenic acids is 3:1.

A repeat of the above experiment with 6·12 g mycelium grown on surface culture gave a total of 1·44 g mixed triterpene acids (23·5%) composed of eburicoic and sulphurenic acids in a 3:1 ratio.

Methyl 3,15-*diacetyl sulphurenate* (IIb)

Methyl sulphurenate (IIa; 100 mg) was acetylated with 0·5 ml anhydrous pyridine and 0·5 ml acetic anhydride for 20 hr at room temp. After removal of the reagents *in vacuo*, the crude residue was crystallized from methanol, m.p. 138–140°; $[\alpha]_D^{23}$ +67° (*c*, 0·41); λ_{max}^{KBr} 5·78, 6·09, 8·05 μ. (Found (average of 3 analyses): C, 73·93; H, 9·73. $C_{36}H_{56}O_6$ (584·81) requires: C, 73·93; H, 9·65%)

Diacetyl sulphurenic acid (IIc)

A solution of 250 mg (0·5 mmoles) methyl sulphurenate (IIa) and 435 mg dried lithium iodide (3·3 mmoles) in 10 ml anhydrous collidine was refluxed under He for 18 hr. The reaction mixture was then poured onto ice and 2N HCl aq and the resulting suspension extracted with methyl isobutyl ketone. The resulting material (153 mg) was acetylated and, after removal of the reagents *in vacuo*, distributed between dil. H_2SO_4 aq and $CHCl_3$. The $CHCl_3$ extracted material (135 mg) was taken

[22] L. F. Fieser and M. Fieser, *Steroids*, p. 94. Reinhold, New York (1959).

up in hexane, a small residue removed by centrifugation and the hexane solution evaporated to dryness. The residue crystallized readily from methanol furnishing 71 mg of IIc, m.p. 234–235°; $[\alpha]_D^{23}$ +58° (*c*, 1·00); λ_{max}^{KBr} 5·76, 5·87, 6·08, 8·04 and 11·28 μ. (Found: C, 73·76; H, 9·43. $C_{35}H_{54}O_6$ requires: C, 73·65; H, 9·54%).

Sulphurenic acid (II)

A suspension of 30 mg IIc in 3 ml 0·7N KOH in methanol was stirred at room temp for 20 hr under a blanket of He. The resulting solution was acidified to pH 2 with 4N H_2SO_4 and the mixture extracted with large volumes of hot methyl isobutyl ketone. The methyl isobutyl ketone extract afforded material, which on crystallization from acetone furnished 13 mg pure II, m.p. 252–254°; $[\alpha]_D^{23}$ +42° (*c*, 0·51 in anhydous pyridine); λ_{max}^{KBr} 2·95, 5·90, 6·08, 11·27 μ. (Found (after drying at 135° for 1/2 hr): C, 76·73; H, 10·18. $C_{31}H_{50}O_4$ (486·71) requires: C, 76·50; H, 10·36%).

The following is a more practical procedure for the preparation of sulphurenic acid from methyl sulphurenate.

Into a solution of 1 g IIa in 50 ml freshly distilled tetrahydrofuran was passed ammonia gas with cooling until a total of 100 ml had condensed. The methyl sulphurenate now had precipitated out and was present as a fine suspension. Small pieces of Li were added with stirring and the flask removed from the cooling bath. The addition of Li was continued until there was a persistent blue color and the ammonia was permitted to evaporate. A small amount of methanol was then added to dissolve the residual Li. The mixture was acidified to pH 2 with dil. H_2SO_4 aq and extracted with methyl isobutyl ketone. The resulting methyl isobutyl ketone extract was extracted with 1N NaOH aq until all the acid had been removed. The aqueous extracts were washed once with methyl isobutyl ketone and acidified with 2N H_2SO_4 to pH 2. The precipitated acids were extracted again with hot methyl isobutyl ketone, the methyl isobutyl ketone extracts washed with water and evaporated to dryness *in vacuo*. The resulting residue (485 mg) which consisted essentially of the very insoluble sulphurenic acid was recrystallized from tetrahydrofuran–acetone and yielded a total of 200 mg pure II, identical in every respect with the acid prepared by the alternate procedure.

Methyl dihydrosulphurenate (III)

To a prereduced suspension of 1·5 g 10% Pd–C in 40 ml ethyl acetate was added a solution of 1·5 g methyl sulphurenate in 100 ml ethyl acetate and the resulting mixture agitated in the presence of H_2 until uptake was complete, which required a total of 2 hr and 30 min. Total uptake: 95 ml H_2; Calcd. for 1 mole: 75 ml. The catalyst was removed by filtration and the solution evaporated to dryness *in vacuo*. The residue on recrystallization from methanol furnished 1·27 g III, m.p. 200–202°; $[\alpha]_D^{23}$ +65° (*c*, 0·46); λ_{max}^{KBr} 2·95, 5·77, 5·84 μ. (Found: C, 76·32; H, 10·87. $C_{32}H_{54}O_4$ (502·75) requires: C, 76·44; H, 10·83%).

Evaporation of the lowest mother liquor furnished an isomer of methyl dihydrosulphurenate (112 mg), isomeric at C-24 with the compound described above, as shown by acetylation to be described in the following experiment.

Methyl diacetyl dihydrosulphurenate (IIIa)

Methyl dihydrosulphurenate (III; 500 mg); was acetylated furnishing after recrystallization from methanol pure IIIa, m.p. 140–142°; $[\alpha]_D^{23}$ +67° (*c*, 0·42); λ_{max}^{KBr} 5·78, 8·05 μ. (Found (after drying at 110° for 4 hr): C, 74·80; H, 9·91. $C_{36}H_{58}O_6$ (586·82) requires C, 73·68; H, 9·96%).

The lowest mother liquor material obtained in the catalytic hydrogenation of methyl sulphurenate (112 mg) was acetylated and the product dissolved in benzene and filtered through a small amount of neutral alumina (activity I). Evaporation of the benzene gave 105 mg of crude diacetate, which was further purified by preparative thin layer chromatography to give the pure 24-isomer of methyl diacetyl dihydrosulphurenate, m.p. 153–155°; $[\alpha]_D^{23}$ +55° (*c*, 0·48); λ_{max}^{KBr} 5·75, 8·05 μ. (Found: C, 73·76; H, 10·06. $C_{36}H_{50}O_6$ (586·2) requires: C, 73·68; H, 9·96%).

Methyl diacetyl 7,11-*diketodihydrosulphurenate* (IV)

To a solution of IIIa (100 mg) in 5 ml glacial acetic acid was added at 70°, dropwise with stirring, a solution of 160 mg CrO_3 in a few drops water and 4 ml glacial acetic acid. Addition of the oxidizing agent was complete after 40 min and the reaction mixture was then cooled and the excess oxidant reduced by the addition by a few drops methanol. The mixture was concentrated to small volume

and taken up in isobutyl methyl ketone and water. The residue obtained from the organic phase (237 mg) was dissolved in 5 ml hexane and chromatographed on 4·7 g neutral alumina, activity I Elution of the column with 200 ml hexane left a small amount of amorphous material. Subsequent elution with benzene–hexane (1:1; 200 ml) followed by benzene alone (250 ml) gave the desired diketone (IV), which after crystallization had m.p. 180–181°; $[\alpha]_D^{23}$ +79° (*c*, 0·41); λ_{max}^{alc} 270 mμ (ε = 9,700); λ_{max}^{KBr} 5·78, 5·96, 8·08 μ. (Found: C, 70·38, H, 8·83. $C_{36}H_{54}O_8$ (614·79) requires: C, 70·33; H, 8·55%).

Methyl $\Delta^{8,24(28)}$-*eburicadiene*-3,15-*dione*-21-*oate* (V)

To a solution of methyl sulphurenate (1 g) in 25 ml acetone was added at 20° dropwise with stirring 1·9 ml Jones reagent (200 mg CrO_3 and 320 mg H_2SO_4 in 1 ml water). After 25 min at 20° methanol was added dropwise to reduce excess CrO_3. After the addition of water and evaporation of the bulk of the acetone *in vacuo* the residue was extracted with $CHCl_3$. The residue obtained from the $CHCl_3$ extract upon recrystallization from methanol furnished the pure diketone V (743 mg) m.p. 141–143°; $[\alpha]_D^{25}$ +83·5° (*c*, 0·40); λ_{max}^{KBr} 5·75, 5·85, 6·08, 11·29 μ. (Found: C, 77·23; H, 9·65. $C_{32}H_{48}O_4$ (496·70) requires: C, 77·37; H, 9·74%).

Methyl 15-*ketoeburicoate* (VI)

To a solution of KBH_4 (100 mg) in 30 ml water and 30 ml dioxane was added at room temp with stirring a solution of 300 mg of V in 30 ml dioxane. The reaction was allowed to proceed for 45 min at room temp, after which time excess KBH_4 was destroyed by the addition of glacial acetic acid. The mixture was taken up in $CHCl_3$, the $CHCl_3$–dioxane phase washed with water, and the solvents evaporated to dryness. Crystallization of the residue from methanol furnished 268 mg material m.p. 130–132° and an additional 120 mg m.p. 118–120°. The analytically pure material had m.p. 130–132° (solvated); $[\alpha]_D^{23}$ +71° (*c*, 0·47); λ_{max}^{KBr} 2·92, 5·77, 6·10, and 11·28 μ. (Found: C 76·66; H, 9·99. $C_{32}H_{50}O_4$ (498·72) requires: C, 77·06; H, 10·11%).

Methyl acetyl 15-*ketoeburicoate* (VIa)

Methyl 15-ketoeburicoate (VI; 240 mg; solvated) on acetylation followed by recrystallization from methanol furnished 217 mg pure acetyl derivative VIa, m.p. 165–166°; $[\alpha]_D^{23}$ +67° (*c*, 1·95); λ_{max}^{KBr} 5·77, 6·10, 8·08, and 11·15 μ. (Found: C, 75·07; H, 9·76. $C_{33}H_{52}O_5$ (528·75) requires: C, 74·96; H, 9·91%).

Methyl 15-*ketodihydroeburicoate* (VIb)

To a prereduced suspension of 80 mg 5% Pd-$BaSO_4$ in 20 ml ethyl acetate was added a solution of 200 mg VI in 20 ml ethyl acetate. The reduction was continued until H_2 uptake was complete (11·0 ml); theory for 1 mole: 10 ml. Recrystallization of the reduction product from methanol gave strongly solvated material (m.p. 144–150°). Recrystallization from acetonitrile gave material, m.p. 144–148°; $[\alpha]_D^{23}$ +62° (*c*, 1·00). (Found: after drying at 100° for 4 hr): C, 76·70; H, 10·41. $C_{32}H_{52}O_4$ (500·74) requires: C, 76·75; H, 10·47%).

Wolff Kishner reduction of methyl acetyl 15-*ketoeburicoate* (VIa) *to methyl acetyl eburicoate* (VII)

Hydrazine hydrate (95%; 10 ml) and 10 g NaOH pellets were refluxed 3 hr and then a sufficient amount of the dried hydrazine was distilled into 10 ml redistilled diethylene glycol, in which 200 mg Na had been dissolved and which had been preheated to 180°, to insure reflux of the mixture at 180°. The mixture was then cooled to room temp and 100 mg vacuum-dried (100°) VIa was added. The resulting solution was refluxed for 18 hr at 180°, after which time hydrazine was distilled off to secure reflux of the solution at 210° (measured in the liquid) for 24 hr. The cooled mixture was acidified with 4N H_2SO_4 and taken up in methyl isobutyl ketone and water. The aqueous phase was extracted several times with methyl isobutyl ketone and the combined extracts evaporated to dryness *in vacuo*. The total residue (95 mg) was taken up in 2 ml methanol and remethylated with ethereal diazomethane for 20 min. After removal of the solvents *in vacuo*, the crystalline residue was recrystallized from methanol, furnishing 47 mg material m.p. 117–125°. This material was further purified by preparative thin layer chromatography on activity V alumina using $CHCl_3$–hexane (1:1) as the liquid phase. The zone possessing an R_f of approximately 0·3 (the first zone from the origin) was eluted

with ethyl acetate and furnished 25 mg crystalline material. This material on recrystallization from methanol gave pure methyl eburicoate, m.p. 114–115°. Identity was confirmed by acetylation, which furnished pure methyl acetyl eburicoate m.p. 151–153°, which gave no depression when mixed with an authentic sample, and whose IR spectrum was identical with that of such a sample.

In addition to the zone of $R_f = 0{\cdot}3$ the above chromatogram showed a zone of $R_f = 0{\cdot}5$. Elution of this zone with ethyl acetate gave 20 mg of crystalline material, which on recrystallization from methanol melted at 169–171°; $[\alpha]_D$ +26° (*c*, 0·47); λ_{max}^{KBr} 3·04, 5·76 μ. In contrast to methyl acetyl eburicoate this compound had no peak at 11·27–11·30 μ characteristic for C = CH_2, nor did it show the NMR signals at 5·27 and 5·33 τ for these vinyl protons.

Acetylation of this material gave a crystalline acetate, m.p. 135–136°; λ_{max}^{KBr} 5·76, 8·05 μ.

Treatment of methyl acetyl eburicoate under Wolff–Kishner conditions

Methyl acetyl eburicoate (100 mg) was treated under Wolff–Kishner conditions exactly ascribed above for the 15-keto derivative. There was isolated after recrystallization from methanol 60 mg crystalline material m.p. 118–123°, which on thin layer chromatography afforded 15 mg methyl eburicoate m.p. 103–112° and 9 mg of the substance m.p. 168–170°, the latter identical by IR spectroscopy with the material isolated in the previous experiment. Identity was confirmed by conversion of the two substances into their acetates, m.p. 151–153° and 135–136°, respectively.

Methyl 14-*methyl*-3-*isopropylidene*-$\Delta^{8,24(28)}$-A-*nor*-5α-*ergostadiene*-15-*one*-21-*oate* (VIII)

To a solution of 47 mg VIb in 15 ml dry toluene, through which a vigorous stream of He gas was passed with stirring at 0°, was added 47 mg PCl_5. After a total reaction time in the dark of 6 min, saturated $NaHCO_3$ aq was added and the mixture stirred for an additional 5 min. The layers were then separated and the toluene extract washed with water, dried (Na_2SO_4) and evaporated to dryness *in vacuo*. The residual material on recrystallization from methanol furnished pure VIII (24 mg) m.p. 131–132°; $[\alpha]_D$ −54°; (*c*, 0·97); λ_{max}^{KBr} 5·76, 6·09 and 11·25 μ; NMR 3 protons each at 8·29 and 8·38 τ $\left(=C\begin{matrix}CH_3\\CH_3\end{matrix}\right)$ (Found: C, 80·00; H, 10·01. $C_{32}H_{48}O_3$ (480·70) requires: C, 79·95; H, 10·07%).

Its 24(28)-dihydro derivative prepared in an analogous fashion had m.p. 144–145°; λ_{max}^{KBr} 5·75 μ.

Methyl 3β, 15α-*dihydroxy*-24-*keto*-Δ^8-*lanostene*-21-*oate* (IX)

A solution of 5 g IIa in 140 ml methylene chloride and 140 ml ethyl acetate was ozonized until the emerging gas liberated I_2 from a KI solution. To the resulting solution was added 5 ml acetic acid and 10 g Zn dust (in portions) and the resulting suspension stirred until it no longer blued starch iodide reagent. The mixture was then filtered and the filtrate extracted with dil. $NaHCO_3$ aq and water. The solvents were removed *in vacuo* and the resulting residue (5 g) dissolved in 200 ml benzene and chromatographed on 250 g neutral alumina. Elution with 9 parts benzene and 1 part $CHCl_3$ (6·6 l.) followed by 300 ml $CHCl_3$–benzene 1:4 produced 3·74 g of material. Crystallization of this material from methanol furnished 1·9 g of the pure 24-keto ester IX, m.p. 179–181°; $[\alpha]_D^{23}$ +56° (*c*, 1·0); λ_{max}^{KBr} 2·82, 2·96, 3·14, 5·81 μ. (Found (after drying for 2 hr at 125°): C, 73·81; H, 9·97. $C_{31}H_{50}O_5$ (502·71) requires: C, 74·06; H, 10·03%).

Methyl 3,15,24-*triketo*-$\Delta^{2'}$-*lanostene*-21-*oate* (X)

A solution of 75 mg IX in 5 ml reagent acetone was oxidized with 1·2 ml Jones CrO_3 reagent containing 20 mg CrO_3 per ml. Methanol was added to the mixture to reduce excess CrO_3 and after the addition of water, the excess methanol was removed *in vacuo*. The steroids were extracted with $CHCl_3$ and the resulting residue recrystallized from methanol to give the pure triketo ester (X; 58 mg) m.p. 176–177°; $[\alpha]_D^{23}$ +79° (*c*, 1·03); λ_{max}^{KBr} 5·76, 5·86 μ. (Found (after drying for 1·5 hr at 125°): C, 74·73; H, 9·40. $C_{31}H_{46}O_5$ (498·68) requires: C, 74·66; H, 9·30%).

Methyl 16,17β-(1′,2′-$\Delta^{2'}$-*cyclohexeno*)-4,4,14-*trimethyl*-3′-*isopropyl*-Δ^8-*androstene*-3,15-*dione*-6′α-*carboxylate* (XI) *and* 3,15,24-triketo-Δ^8-*lanostene*-21-*oic acid* (Xa)

To a solution of 500 mg K metal in 100 ml dry, redistilled t-butanol was added at room temp 500 mg X. The mixture was stirred under N_2 at room temp for 2 hr and 20 min, after which time

water was added and the mixture was neutralized by the addition of glacial acetic acid. The solution was concentrated to remove most of the t-butanol and extracted with $CHCl_3$. The $CHCl_3$ extract was evaporated to dryness *in vacuo*, and the residue taken up in ether, whereupon crystallization occurred. The crystals were removed by centrifugation and recrystallized from acetone to furnish pure Xa, m.p. 264–268°; $[\alpha]_D^{28}$ +81° (*c*, 1·03); λ_{max}^{KBr} 3·05, 5·76 and 5·87 μ. The infrared spectrum of this substance in KBr indicated that it was essentially in the pseudo acid form. It also represents the only derivative reported in this paper, in which the peak for the 15-keto group (5·76 μ) is not superimposed on the ester carbonyl peak. (Found: C, 74·45; H, 9·23. $C_{30}H_{44}O_5$ requires: C, 74·34; H, 9·15%).

Methylation of the acid with diazomethane afforded the starting methyl ester X, m.p. 173–174°.

The ether mother liquor from the triketolanostenoic acid (Xa) on methylation with excess diazomethane furnished crystalline material, which was recrystallized first from ether and finally from methanol. There was obtained 45 mg of the methyl ester XI, m.p. 184–187°; $[\alpha]_D^{28}$ +89° (*c*, 0·31); λ_{max}^{alc} 251 mμ (ε = 13,250), 356 mμ (ε = 80, shoulder), 364 mμ (ε = 65); λ_{max}^{KBr} 5·77, 5·88 and 6·18 μ. (Found: C, 77·48; H, 9·28. $C_{31}H_{44}O_4$ requires: C, 77·46; H, 9·23%).

The mother liquors from the crystallization of the above methyl ester XI were evaporated to dryness *in vacuo*, and the total residue (290 mg) dissolved in 5 ml benzene and 50 ml hexane and the solution chromatographed on 10 g neutral alumina. Elution of the column with benzene–hexane 1:1 eluted in the first 600 ml 52 mg crystalline material, which after recrystallization from methanol, m.p. 183–185° (33 mg) was identical in all respects with the material described above. Continued elution of the column with the same solvent mixture (1,000 ml) eluted 100 mg crude material, which was purified by preparative thin layer chromatography on activity V alumina using $CHCl_3$ as the developing agent. There was isolated 33 mg of crystalline material which after one recrystallization from methanol furnished an additional 24 mg methyl ester XI m.p. 181–183°. The total yield from all these sources was 102 mg or 22% of theory.

3β,15α-Dihydroxy-24-keto-Δ⁸-lanostene-21-oic acid (IXa)

A solution of 500 mg IX in 50 ml oxygen-free KOH solution prepared by mixing 72 ml 5% ethanolic KOH with 4·6 ml water was refluxed under a gentle stream of He for 6 hr. The mixture was cooled, diluted with water and the alcohol evaporated *in vacuo*. Acidification with glacial acetic acid gave a suspension which was extracted with methyl isobutyl ketone. The residue (500 mg) on recrystallization from methanol furnished pure IXa (345 mg), m.p. 265–267°; $[\alpha]_D^{28}$ +58° (*c*, 0·26 in 95% ethanol); λ_{max}^{KBr} 2·95, 5·86 μ. (Found (after drying for 18 hr at 100°); C, 71·36; H, 9·71. $C_{30}H_{48}O_5 \cdot H_2O$ requires: C, 71·11; H, 9·55%. Found (after drying to constant weight at 150°): C, 73·85; H, 9·96. $C_{30}H_{48}O_5$ (488·68) requires: C, 73·73; H, 9·90%).

Methyl 3β,15α,24-trihydroxy-Δ⁸-lanostene-21-oate (XX)

To a solution of 30 mg KBH_4 in 3 ml water and 3 ml dioxane was added 30 mg IX in 3 ml dioxane. The mixture was allowed to remain at room temp for 45 min at which time excess KBH_4 was destroyed by the addition of acetic acid. The mixture was extracted with $CHCl_3$ and water, the $CHCl_3$–dioxane solution was washed with water and evaporated to dryness *in vacuo*. The residual trihydroxy acid methyl ester XX after recrystallization from acetone–hexane had m.p. 185–186°; $[\alpha]_D^{25}$ +57° (*c*, 1·22); λ_{max}^{KBr} 2·92, 5·80 μ. (Found: C, 74·74; H, 10·44. $C_{31}H_{52}O_5$ (504·73) requires: C, 73·76; H, 10·38%).

Hydrolysis of methyl 3β,15α,24ξ-trihydroxy-Δ⁸-lanostene-21-oate with KOH *in ethanol*

A solution of 8 mg methyl 3β, 15α, 24ξ-trihydroxy-Δ^8-lanostene-21-oate in 3·5 ml of a mixture consisting of 72 ml 5% ethanolic KOH (O_2-free) and 4·6 ml of water was refluxed under He for 5 hr. The solution was acidified with glacial acetic acid and extracted with methyl isobutyl ketone. After removal of the solvent *in vacuo* there remained crystalline 3β,15α,24ξ-trihydroxy-Δ^8-lanostene-21-oic acid.

Treatment of 3β-acetoxy-Δ⁸,²³-lanostadiene-24-ol-21-oic acid 21 → 24-*lactone* (XVIII) *with* KOH *in ethanol*

A solution of 100 mg XVIII[24] in 30 ml of a mixture of 72 ml O_2-free 5% ethanolic KOH and 4·6 ml of water was heated under reflux for 6 hr. The cooled solution was diluted with water and the

bulk of the ethanol removed *in vacuo*. The resulting suspension was extracted with $CHCl_3$ to obtain the neutral constituents of the mixture. There remained 46 mg of material, which after crystallization from methanol gave 35 mg pure XIXb, m.p. 158°; $[\alpha]_D$ +31° (*c*, 0·65); λ_{max}^{KBr} 2·8–3·1, 5·80 (shoulder), 5·85 μ; NMR 5·87τ (quartet, 7) and 8·80τ (triplet, 7) (CH_3CH_2O). (Found: C, 76·67; H, 10·40. $C_{32}H_{52}O_4$ requires: C, 76·75; H, 10·47%).

The $CHCl_3$-extracted aqueous solution was acidified to pH 2·0 with HCl aq and extracted with methyl isobutyl ketone. The dried methyl isobutyl ketone extract on evaporation left 42 mg acidic material which on crystallization from methanol afforded 27 mg XIX, m.p. 264–267°; $[\alpha]_D$ +34° (*c*, 0·42); λ_{max}^{KBr} 2·95, 5·85 μ. (Found (after drying at 140°): C, 76·54; H, 10·42. $C_{30}H_{48}O_4$ requires: C, 76·22; H, 10·24%).

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