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# THE STRUCTURE AND SOME ASPECTS OF THE BIOSYNTHESIS OF PLEUROMUTILIN

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**Abstract**—Evidence is presented which confirms the structure I for pleuromutilin. The specific incorporation of [$^{14}$C] acetic and mevalonic acids verifies its diterpenoid nature, and some aspects of its stereochemistry are discussed.

PLEUROMUTILIN is an antibiotic inhibitory to gram-positive bacteria isolated from the Basidiomycetes *Pleurotus mutilus,*[1] *P. Passeckerianus,*[1] and *Drosophila subatrata.*[2] We were initially led to study its structure through an interest in fungal terpenes. Through the kindness of Dr. M. Anchel, who had carried out a preliminary chemical study[3] of the compound, we were provided with a culture of *P. mutilus*, and were able with some difficulty to produce limited quantities of the antibiotic. Structural work in Manchester on pleuromutilin was commenced in 1959 by Dr. D. W. Cameron,[4] and then continued in 1961–63 by Dr. C. W. Holzapfel.[5] Our work was well advanced, defining the presence of a perhydroindanone nucleus and attached medium-sized ring, and the nature and relative situations of most of the substituents, when we were informed by Dr. D. Arigoni that his independent work led to the structure I for pleuromutilin. We continued only long enough to complete aspects of our own experimental approach which provided independent confirmation of his structure I, except for the position of the secondary Me group on the cyclohexane ring. In order to avoid further duplication, we then abandoned further experimental work, notably on the stereochemistry and biosynthesis.[6–9] We discuss here our own work, which differs considerably in detail from that of Dr. Arigoni, reported[9] to date only in outline without experimental detail. Through the courtesy of Dr. Arigoni, his complete publication now appears in the Swiss journal concurrently with this report. To avoid complication, we use here his nomenclature and numbering system.[9]

The initial studies by Anchel[3] defined pleuromutilin as a neutral substance, $C_{22}H_{34}O_5$, with two alcoholic OH groups and a hindred carbonyl function. The two remaining oxygens were thought to be in an ester or lactone, since alkaline hydrolysis

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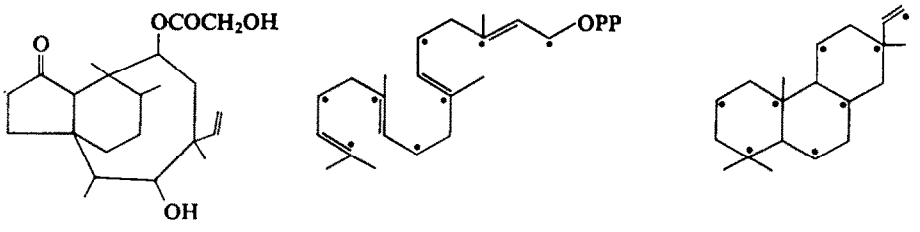
[1] F. Kavanagh, A. Hervey and W. J. Robbins, *Proc. natn. Acad. Sci. U.S.A.* **37**, 570 (1951).
[2] F. Kavanagh, A. Hervey and W. J. Robbins, *Proc. natn. Acad. Sci. U.S.A.* **38**, 555 (1952).
[3] M. Anchel, *J. biol. Chem.* **199**, 133 (1952).
[4] D. W. Cameron, Ph.D. Thesis, Manchester (1960).
[5] C. W. Holzapfel, Ph.D. Thesis, Manchester (1963).
[6] A. J. Birch, D. W. Cameron, C. W. Holzapfel and R. W. Rickards, *Chem. & Ind.* 374 (1963).
[7] L. Ružička, in *The Chemistry of Natural Products* 2; *Second International Symposium on the Chemistry of Natural Products*, p. 493. Butterworths, London (1963).
[8] D. Arigoni, in *Biogenesi delle Sostanze Naturali*; VII° *Corso Estivo di Chimica* p. 1. Accad. naz. Lincei, Roma (1964).
[9] D. Arigoni, *Gazz. Chim. Ital.* **92**, 884 (1962).

gave a neutral compound, $C_{20}H_{32}O_3$, herein called mutilin (previously[6] named pleuromutenol). Preliminary work[4] showed the cleaved $C_2$ fragment to be glycollic acid, and since mutilin carried the expected carbonyl and two OH groups, pleuromutilin is therefore a glycollic ester of mutilin. That no fundamental change had occurred on hydrolysis was apparent from the similarity of the ORD curves of the starting material and product.

In addition to other data to be discussed, maxima at 3080, 1642 and 914 $cm^{-1}$ in the IR spectrum of mutilin (in Nujol) indicated the presence of a vinyl group, which could be hydrogenated to the dihydro derivative, $C_{20}H_{34}O_3$, with loss of these bands This latter compound contained no further unsaturation, as shown by its negative tetranitromethane reaction and stability to ozone, and so must be tricarbocyclic. The $C_{20}$ formula, the number of rings, and the presence of a vinyl group suggested that mutilin itself is a diterpene, derived[10] by cyclization of a precursor of the geranyl-geraniol type II. Cameron[4,6] therefore investigated the *in vivo* incorporation into pleuromutilin of [2-$^{14}$C]mevalonic lactone, a specific terpene precursor, which occurred exclusively into the $C_{20}$ nucleus to the extent of 5·5%, and of [1-$^{14}$C]acetic acid, which was also effectively utilized (1·7%). [1-$^{14}$C]Acetic acid would label the $C_{20}$ precursor II in eight places (* indicates $^{14}$C), and after cyclization to a tricyclic diterpene the methylene carbon of the resulting vinyl group should contain one eighth of the total radioactivity, as can be seen from the typical diterpene skeleton III.[10] This was confirmed quantitatively for pleuromutilin (relative molar activity[11] $44{\cdot}3 \times 10^4$) by ozonolysis of mutilin




I II III

(r.m.a. $41{\cdot}6 \times 10^4$) to give formaldehyde (r.m.a. $5{\cdot}16 \times 10^4$) and a nor-aldehyde $C_{19}H_{30}O_4$ (r.m.a. $36{\cdot}1 \times 10^4$), which carried one-eighth and seven-eighths respectively of the activity of mutilin. Consequently, the mutilin nucleus was known[4] to be diterpenoid before any detailed structural conclusions were reached as to the nature of the ring system. This knowledge was of considerable value in subsequent structural work, further discussion of which is continued on a logical rather than chronological basis.

*Oxidation-reduction reactions of the oxygenated substituents*

Pleuromutilin shows OH absorption in the IR at 3550 $cm^{-1}$ (in $CS_2$) together with a max at 1736 $cm^{-1}$ which represents a combination of bands due to the glycollic ester and the carbonyl function. That this latter is a cyclopentanone is supported by the characteristic band at 1735 $cm^{-1}$ in the spectrum* of mutilin. Chromium trioxide oxidation of mutilin in acetone gave a trione, $C_{20}H_{28}O_3$, indicating that the two OH groups

*IR spectra refer to $CCl_4$ sols unless otherwise stated.

[10] A. J. Birch, R. W. Rickards, H. Smith, A. Harris and W. B. Whalley, *Tetrahedron* 7, 241 (1959).
[11] A. J. Birch, R. A. Massy-Westropp, R. W. Rickards and H. Smith, *J. Chem. Soc.* 360 (1958).

were both secondary; the IR spectrum of the trione, $\nu_{max}$ 1702 and 1740 $cm^{-1}$, indicated the formation of other types of carbonyl than cyclopentanone.

Further work required the ability to distinguish, by selective reactions, the oxygenated substituents, in particular the two OH functions of mutilin. Acetic anhydride in pyridine preferentially attacked the primary (glycollic ester) OH of pleuromutilin, affording a mono-acetate $C_{24}H_{36}O_6$, together with some diacetate. Oxidation of the mono-acetate with chromium trioxide, followed by hydrolysis, gave a hydroxy-A-dione, $C_{20}H_{30}O_3$, $\nu_{max}$ 3655, 1740 and 1702 $cm^{-1}$. The same hydroxydione could be obtained by direct oxidation of pleuromutilin (to the diketoglycollate $C_{22}H_{32}O_5$ as the major product, together with some diketo-oxalate) followed by hydrolysis, or even as the main product of treatment of mutilin itself with one mole of oxidant.

An isomeric hydroxy-B-dione, in which the other OH of mutilin is free, could be obtained by the following sequence. Selective mono-acetylation of mutilin was readily effected by acetic anhydride in pyridine at room temperature, significant yields of the diacetate resulting only after prolonged heating. The keto-mono-acetate, $C_{22}H_{34}O_4$, afforded after oxidation to a diketo-acetate $C_{22}H_{32}O_4$ and saponification, the hydroxy-B-dione, $C_{20}H_{30}O_3$, $\nu_{max}$ 3570, 1737 and 1698 $cm^{-1}$. Reacetylation of this last compound gave back the previous acetate, indicating that no epimerization had occurred during hydrolysis.

There is therefore a considerable difference in reactivity, presumably steric in origin, between the OH functions of mutilin. Hydroxyl-A, which is esterified in pleuromutilin itself, is unreactive to both esterification and oxidation compared to hydroxyl-B, the free nuclear OH of pleuromutilin.

Mutilin reacted very slowly with $NaBH_4$, but was reduced by LAH stereoselectively to a triol $C_{20}H_{34}O_3$. Lithium and methanol in liquid ammonia reduced both the vinyl and carbonyl groups of mutilin giving two isomeric triols $C_{20}H_{36}O_3$, the minor and less polar one being identical with the hydrogenation product of the triol from hydride reduction. These results are rationalized on the basis of the hindered nature of the cyclopentanone carbonyl, resulting in steric approach control[12] in the metal hydride reduction, giving probably the less stable epimer. In the dissolving metal reduction[13], both steric approach and product control factors must be involved, leading to a mixture of epimers in which probably the more stable predominates. This major product afforded a triacetate, and could be oxidized to a trione identical with that from hydrogenation of mutilin trione.

*The vinyl and methyl substituents*

The PMR spectrum* of mutilin clearly shows that the vinyl group is attached to a quaternary carbon, the methine proton appearing as a quartet of sharp lines centred at $\tau$ 3·77 coupled only to the vinylic methylene protons ($J_{cis}$ = 11 c/s; $J_{trans}$ = 18 c/s). Each of the methylene protons gives a quartet, centred at $\tau$ 4·64 and 4·70 respectively, since in addition to coupling with the methine proton they are mutually coupled ($J_{vic}$ = 1·5 c/s).

*PMR spectra were recorded at 60 Mc/s for solns in $CDCl_3$ (except where otherwise stated), using TMS as internal reference.

[12] W. G. Dauben, G. J. Fonken and D. S. Noyce, *J. Amer. Chem. Soc.* **78**, 2579 (1956); W. G. Dauben, E. J. Blanz, J. Jiu and R. A. Micheli, *Ibid.* **78**, 3752 (1956).

[13] Cf. H. Smith, *Organic Reactions in Liquid Ammonia* p. 217. Interscience, New York (1963).

This vinyl group was readily reduced catalytically in pleuromutilin itself or its various derivatives to the corresponding dihydro compounds. Reduction of the vinyl group of mutilin by lithium and methanol in ammonia (see above), although a well-known type of reaction,[14] is particularly facile in this case and probably indicates that the vinyl is not notably sterically hindered, since solvation of intermediate ions is crucial in this process. Another contributing factor could be the proximity of an OH group (see below).

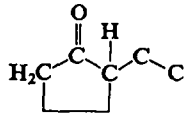
Singlets at $\tau$ 8·56 and 8·82 and doublets centred at $\tau$ 9·10 and 9·28 ($J=6$ and 7 c/s respectively) in the PMR spectrum of pleuromutilin indicate two tertiary and two secondary C-Me groups. Oxidation of the secondary OH groups of mutilin does not generate a $CH_3CO$– system, since all the C-methyl resonances of the resulting trione occur in the vicinity of $\tau$ 8·5.[15] Together with the fact that Kuhn-Roth oxidation of mutilin gave no higher volatile acids than acetic acid, this implies that all the Me groups are directly attached to the ring system.

*The environment of the cyclopentanone group*

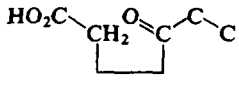
The IR spectrum of mutilin has a max at 1420 $cm^{-1}$ indicating the probable presence of the grouping –$COCH_2$–, which was supported by a strong positive colour test with *m*-dinitrobenzene in alkali[16] and confirmed by the production of an hydroxymethylene derivative $C_{21}H_{32}O_4$, $\lambda_{max}$ 268 m$\mu$ ($\epsilon$ 8600) shifted by alkali to $\lambda_{max}$ 308 m$\mu$ ($\epsilon$ 20,600). Treatment of this derivative first with alkaline peroxide and then with chromium trioxide (to oxidize the two OH functions) gave a diketodicarboxylic acid, converted on heating into a diketo-anhydride, $\nu_{max}$ (in chf) 1702 (ketones), 1756 and 1802 $cm^{-1}$ (anhydride). This is clearly the glutaric anhydride[17] expected if the original CO is, as suggested by IR spectra, a cyclopentanone.

Further exploration of the CO environment was achieved by various oxidation reactions.

Diacetylmutilin reacted rapidly with one mole of oxygen in the presence of potassium t-butoxide[18] to give a diacetoxyketocarboxylic acid, $C_{24}H_{36}O_7$, $\nu_{max}$ (in Nujol) 3500–2600 (carboxyl), 1724 (acetates and ketone), and 1689 $cm^{-1}$ (carboxyl). The PMR spectrum of this product showed the acetoxyl resonances at $\tau$ 7·96 and 8·00, but no methyl ketone or aldehyde function. Consequently the reaction involves cleavage of a system –$COCH<$, and the combined evidence leads to the cyclopentanone environment IV in mutilin. The ring-opened carboxylic acid V will be further discussed below.



IV



V

[14] H. Smith, *Organic Reactions in Liquid Ammonia* p. 212. Interscience, New York (1963).

[15] L. M. Jackman, *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry* p. 57. Pergamon Press, Oxford (1959).

[16] W. Zimmermann, *Z. phyziol. Chem.* **245**, 47 (1936).

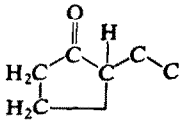
[17] L. J. Bellamy, *The Infrared Spectra of Complex Molecules* p. 127. Methuen, London (1958).

[18] W. von E. Doering and R. M. Haines, *J. Amer. Chem. Soc.* **76**, 482 (1954); E. Elkik, *Bull. Soc. Chim. Fr.* 933 (1959); B. Camerino, B. Patelli and R. Sciaky, *Tetrahedron Letters* No. 16, 554 (1961); E. J. Bailey, D. H. R. Barton, J. Elks and J. F. Templeton, *J. Chem. Soc.* 1578 (1962); and Refs cited therein.

Treatment of diacetylmutilin with selenium dioxide in acetic acid gave two products, one of which, a diketodiacetate $C_{24}H_{34}O_6$, was clearly a normal hydroxide-soluble diosphenol with $\lambda_{max}$ 268 m$\mu$ ($\epsilon$ 12,000) changed by alkali to $\lambda_{max}$ 309 m$\mu$ ($\epsilon$ 8900).[19] The second was a triketodiacetate, $C_{24}H_{32}O_7$, sufficiently acidic to dissolve in aqueous sodium carbonate and reducible with zinc and acetic acid to the normal diosphenol. These properties, together with an UV maximum at 343 m$\mu$ ($\epsilon$ 4600) shifted reversibly by alkali to 443 m$\mu$ ($\epsilon$ 3700), indicate an enolised 1, 2, 3-trione system.

Similar oxidation of dihydromutilin-trione gave in an analogous manner a normal diosphenol, now a tetraone $C_{20}H_{28}O_4$ [$\lambda_{max}$ 269 m$\mu$ ($\epsilon$ 12,700) changing in alkali to $\lambda_{max}$ 302 m$\mu$ ($\epsilon$ 7500)], and a more acidic pentaone $C_{20}H_{26}O_5$ [$\lambda_{max}$ 355 m$\mu$ ($\epsilon$ 4700) changing reversibly in alkali to $\lambda_{max}$ 443 m$\mu$ ($\epsilon$ 4100)], which were interconvertible by selenium dioxide oxidation and zinc–acid reduction.

These results necessitate the environment VI for the cyclopentanone in pleuromutilin, which can be oxidized via the diosphenols VII to the cyclopentan-1,2,3-triones VIII without skeletal rearrangement. That the tetraone, and hence probably also the



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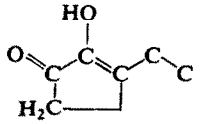
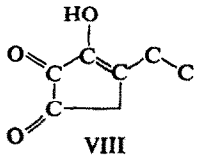


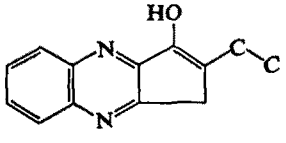
VII



VIII

analogous diketodiacetate, have the diosphenol system enolised towards the methine hydrogen (as in VII) rather than towards the methylene group follows from the absence of olefinic proton absorption in the $\tau$ 3–5 region of the PMR spectrum.[20] The singlet resonance at $\tau$ 3·76 is that of the enolic OH, being removed immediately by deuterium exchange. The pentaone (in pyridine) also lacks olefinic PMR absorption, and, as later evidence will prove, both 1,2,3-triones VIII can only enolize in the direction shown. IR absorption in the 1700–1730 $cm^{-1}$ carbonyl region of all these selenium dioxide oxidation products is complex; the two cyclopentan-1,2,3-triones (VIII), however, show an additional max (in Nujol) at 1760 $cm^{-1}$ corresponding to the new cross-conjugated CO group in the strained five-membered ring (cf. the analogous case of bisnorfriedelenedione,[21] $\nu_{max}$ 1764 $cm^{-1}$).

Under conditions which left the tetraone itself unchanged, the pentaone reacted with *o*-phenylenediamine to give the expected quinoxaline, $C_{26}H_{30}N_2O_3$, which must have the enolic partial structure IX. The derivative was still soluble in alkali, and



IX

showed OH absorption in the IR at 3450 $cm^{-1}$ which was removed by conversion to the enol acetate, $\nu_{max}$ (in $CS_2$) 1771 $cm^{-1}$.

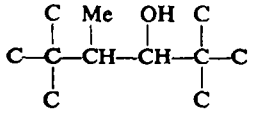
[19] A. I. Scott, *Interpretation of the Ultraviolet Spectra of Natural Products* p. 60. Pergamon Press, Oxford (1964).

[20] C. R. Noller, A. Melera, M. Gut, J. N. Shoolery and L. F. Johnson, *Tetrahedron Letters* No. 15, 15 (1960).

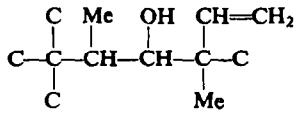
[21] G. Brownlie, F. S. Spring and R. Stevenson, *J. Chem. Soc.* 216 (1959).

*The environment of hydroxyl-B (free in pleuromutilin)*

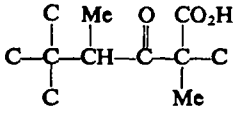
Acetylation of hydroxyl-B as in the acetyl derivative of hydroxy-B-dione gives rise to PMR absorption characteristic of the A proton of the AX system $-CH(OCOR)CH<$, a sharp one-proton doublet ($J=6{\cdot}5$ c/s) at $\tau$ 4·95. PMR spectra of mutilin derivatives in which this hydroxyl-B is oxidized to CO, such as the diketoglycollate and its hydrolysis product hydroxy-A-dione, show a 1:3:3:1 quartet centred at $\tau$ 6·72 corresponding to the A proton of an $AX_3$ system, which is absent from the spectrum of mutilin itself. In each case the quartet splitting (7 c/s) is the same as that of a doublet due to one of the secondary Me groups. Unless the stereochemistry of the system is such as to preclude observable spin coupling to other neighbouring protons, these spectroscopic data suggest the environment X for hydroxyl-B of mutilin.



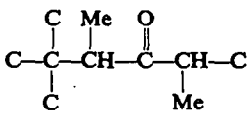
X



XI



XII



XIII

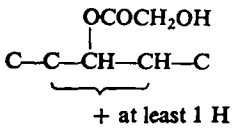
When the hydroxy-A-dione, with hydroxyl-B oxidized to CO, was successively ozonized and oxidized with hydrogen peroxide, a non-crystalline acid was produced, the IR spectrum of which, with $\nu_{max}$ 3480 (hydroxyl-A), 3500–2600 (carboxyl), 1732 (cyclopentanone), and 1702 $cm^{-1}$ (carboxyl and ketone-B), was in accord with the expected nor-hydroxy-A-dionecarboxylic acid. This material had the properties of a $\beta$-keto-acid, and decarboxylated in hot aqueous sodium carbonate solution or on heating alone to 130°. In the latter case the neutral product appeared to be a mixture of epimers of the expected bisnor-hydroxy-A-dione, $C_{18}H_{28}O_3$, one of which, $\nu_{max}$ 3625 (hydroxyl-A), 1739 (cyclopentanone), and 1700 $cm^{-1}$ (ketone-B), was obtained crystalline by chromatography.

The analogous nor-acid $C_{19}H_{30}O_5$, $\nu_{max}$ (in Nujol) 3615 and 3317 (hydroxyls), 3500–2500 and 1708 (carboxyl), and 1736 $cm^{-1}$ (cyclopentanone), from mutilin itself is stable under identical conditions, and hence ketone-B rather than the cyclopentanone must be responsible for the decarboxylation. The vinyl group is therefore attached in the $\alpha$-position to the oxygen function-B, and it has been noted that it is on a quaternary carbon atom. Since mutilin is known to be a diterpene, biogenetic considerations (cf. II and III, and see below) suggest that this carbon probably also carries a (tertiary) Me group. This expectation is supported by PMR evidence. The above bisnor-hydroxy-A-dione in which one of the two tertiary Me groups of mutilin should now be secondary, in fact contains only one tertiary Me singlet at $\tau$ 8·61, together with secondary Me resonances overlapping at higher field. The region of mutilin near oxygen-B can now be expanded from X to XI, whilst XII and XIII represent the above $C_{19}$-nor and $C_{18}$-bisnor compounds respectively.

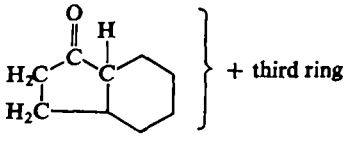
The carbonyl-B was found to be relatively unreactive towards oxygen in the presence of t-butoxide. The main product from treatment of the acetate of hydroxy-A-dione under these conditions was an acetoxydiketocarboxylic acid $C_{22}H_{32}O_6$, $\nu_{max}$ (in Nujol) 3200–2500 (carboxyl), 1730 (acetate), and 1708 $cm^{-1}$ (carboxyl and carbonyls), in which the presence of a PMR quartet at $\tau$ 6·64 (1:3:3:1, $J$=6·5 c/s) due to the –C*H*MeCO– system showed that the cyclopentanone had cleaved preferentially.

*The environment of hydroxyl-*A *(esterified in pleuromutilin)*

The methine proton of the –C*H*(OCOCH$_2$OH)– system of pleuromutilin itself resonates at $\tau$ 4·15 primarily as a doublet, $J$=8 c/s, each component of which is considerably broadened. Similar patterns occur in the related diketoglycollate and in mutilin diacetate at $\tau$ 3·94 and 4·33 respectively, and confirm that this methine proton is strongly spin-coupled to one adjacent nucleus and weakly coupled to at least one other, as in the partial structure XIV.




XIV




XV

*The ring system: general evidence*

The dihydrotriol from lithium-ammonia reduction of mutilin was converted into its triacetate and dehydrogenated with selenium at 300° for 17 hr. Chromatography of the resulting hydrocarbons separated material with weak benzenoid UV absorption from a fluorenoid fraction. Rechromatography of the latter afforded a crystalline fluorene, $C_{18}H_{20}$, $\lambda_{max}$ (in hexane) 261, 271, and 301 m$\mu$ ($\epsilon$ 18,600, 16,600, and 4000),[22] probably an ethyltrimethylfluorene from its mass spectrum* in which the base peak at *m/e* 221 corresponded to the loss of a Me radical[23] from the molecular ion at *m/e* 236. The non-crystalline fluorenes were oxidized with permanganate in acetone to a mixture of yellow fluorenones, $\lambda_{max}$ 256 and 264 m$\mu$ ($\epsilon$ 57,000 and 72,000),[22] shown by mass spectrometry to comprise $C_{20}H_{22}O$, $C_{19}H_{20}O$, and $C_{18}H_{18}O$ components giving rise to molecular ions at *m/e* 278, 264, and 250 with corresponding decarbonylated fragment ions[24] at *m/e* 250, 236, and 222.

This dehydrogenation to alkylfluorenes in moderate (4%) yield was puzzling in view of the proven substitution VI of the cyclopentanone ring, which excludes a hydrofluorene skeleton for mutilin. However, if the nucleus were that of a hydroindane to which an additional ring was attached, as in XV, rearrangement during dehydrogenation could yield the aromatic fluorene system, whilst indenes which would be expected to arise directly from such a system would polymerize[25] under the reaction conditions.†

*We thank Professor C. Djerassi for mass spectra of these fluorene derivatives.

†This dehydrogenation of triacetyl dihydromutilin triol to fluorenes contrasts markedly with the selenium dehydrogenation of mutilin itself to 6,7-dimethylindan-1-one reported by Dr. Arigoni.[9]

[22] Cf. D. M. W. Anderson, N. Campbell, D. Leaver and W. H. Stafford, *J. Chem. Soc.* 3992 (1959); E. Lippert and H. Walter, *Angew. Chem.* **71**, 429 (1959).

[23] Cf. H. Budzikiewicz, C. Djerassi and D. H. Williams, *Interpretation of Mass Spectra of Organic Compounds* p. 166. Holden-Day, San Francisco (1964).

[24] J. H. Beynon, *Mass Spectrometry and its Applications to Organic Chemistry* p. 260. Elsevier, Amsterdam (1960).

[25] G. S. Whitby and M. Katz, *J. Amer. chem. Soc.* **50**, 1160 (1928).

The presence of a hydroindane nucleus in mutilin was supported by mass spectroscopic evidence.* Thus, spectra of both mutilin ($M^+$ 320) and the corresponding trione ($M^+$ 316) showed base peaks at *m/e* 163, $C_{11}H_{15}O^+$, corresponding to a trihydrodimethylindanone ion. To confirm this peak assignment mutilin was reduced under Huang-Minlon conditions to deoxy-mutilin, $C_{20}H_{34}O_2$, $\nu_{max}$ (in $CS_2$) 3648 $cm^{-1}$ (hydroxyls), which was then oxidized to the corresponding deoxydione, $C_{20}H_{30}O_2$, $\nu_{max}$ 1697 $cm^{-1}$. The mass spectrum of this derivative ($M^+$ 302) lacking the cyclopentanone carbonyl now showed the base peak at *m/e* 149, $C_{11}H_{17}^+$, corresponding to a trihydrodimethylindane ion. In all these spectra, cleavage of the third ring from the indane nucleus is accompanied by transfer of one hydrogen atom from the charged indane moiety.

Proof that the third carbocyclic ring of mutilin was in fact medium-sized followed from the study of the relations between the oxygen functions, and from some of their reactions. However, it was apparent early in the work that CO functions derived from the OH groups A and B of mutilin were very unreactive towards reagents such as 2,4-dinitrophenylhydrazine and phenyl magnesium iodide, probably more so than their respective environments XIV and XI would warrant, whilst their IR absorption coefficients were considerably less than that of the cyclopentanone. These facts suggested that the two groups might be in a medium-ring, which is known to decrease reactivity[26] and absorption intensity[27] of carbonyl functions.

*Relations between the oxygen functions: The medium ring*

Mutilin hydroxy-A-dione with refluxing methanolic potassium hydroxide gave a non-crystalline hydroxy-$\alpha\beta$-unsaturated ketone, $\lambda_{max}$ 236 m$\mu$ ($\epsilon$ 13,500), $\nu_{max}$ 3480 (hydroxyl), 1696 and 1626 $cm^{-1}$ (conjugated ketone), with the vinyl group intact ($\nu_{max}$ 3075, 1626, and 910 $cm^{-1}$) but lacking the saturated five-ring carbonyl. The reaction was clearly of the retro-Michael type, but reliable analyses of the product could not be obtained (probably due to its ease of dehydration, see below). However, it reacted with 2,4-dinitrophenylhydrazine in methanolic acid to yield a derivative, $C_{27}H_{36}N_4O_6$, containing one MeO group and lacking OH or CO functions. This formation of a mono- rather than a bis-derivative, together with O-methylation, indicates the presence of a stable cyclic hemiketal structure in the hydroxydione, $C_{20}H_{30}O_3$, expected from a retro-Michael sequence. A 1,5-relationship between the cyclopentanone CO and the CO function B is thus established.

The hemiketal gave propionic as well as acetic acid on controlled Kuhn-Roth oxidation,[28] indicating the presence of a C-ethyl group which must have been generated in the retro-Michael fission. The compound was stable towards chromium trioxide under basic conditions in pyridine, but was readily oxidized by the reagent in acetone, affording an OH-free $\gamma$-lactone, $C_{18}H_{24}O_3$, $\nu_{max}$ 1773 $cm^{-1}$, which still contained the $\alpha\beta$-unsaturated ketone, $\nu_{max}$ 1696 and 1625 $cm^{-1}$, $\lambda_{max}$ 236 m$\mu$ ($\epsilon$ 14,000). This $\gamma$-lactone dissolved slowly in aqueous alkali, from which it was recovered on acidification. Its PMR spectrum showed, in addition to the low-field absorption of the $-CO_2CH-$ and

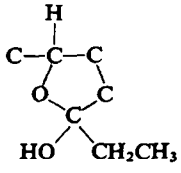
*We are indebted to Dr. J. H. Beynon and Dr. R. I. Reed for these spectra.

[26] V. Prelog, *J. Chem. Soc.* 420 (1950); H. C. Brown, R. S. Fletcher and R. B. Johannesen, *J. Amer. Chem. Soc.* 73, 212 (1951); S. L. Friess and P. E. Frankenburg, *Ibid.* 74, 2679 (1952).

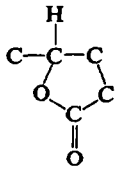
[27] T. Bürer and Hs. H. Günthard, *Helv. Chim. Acta* 39, 356 (1956).

[28] C. F. Garbers, H. Schmid and P. Karrer, *Helv. Chim. Acta* 37, 1336 (1954).

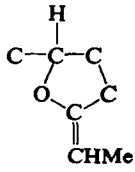
vinyl protons, only three C-Me groups, two tertiary (s, $\tau$ 8·64 and 8·73) and one secondary (d, $\tau$ 9·00, $J=7$ c/s). These data, together with the $C_{18}$ formula and the fact that Kuhn-Roth oxidation[28] no longer gave propionic acid, indicated oxidative loss of the Et group from the cyclic hemiketal system XVI to form the $\gamma$-lactone XVII. Similar processes are known,[29] the oxidation intermediate[29,30] being probably the enol ether



XVI



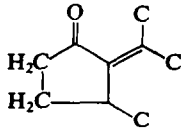
XVII



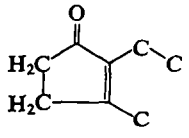
XVIII

XVIII. This dehydration product XVIII could be prepared from the hemiketal XVI by warming with acetic anhydride; its structure follows from ozonolysis to equimolar amounts of acetaldehyde and formaldehyde (from the vinyl group), and, as anticipated, it was oxidized by Jones' reagent to the $\gamma$-lactone XVII in high yield. Ozonolysis of the hemiketal itself gave formaldehyde together with small, variable quantities of acetaldehyde, indicating contamination with some dehydrated material XVIII, which may explain the poor analytical figures referred to earlier.

We turn now to evidence concerning the fate of the cyclopentanone itself in the retro-Michael reaction. The $\alpha\beta$-unsaturated CO chromophore in the hemiketal product or the derived $\gamma$-lactone was resistant to catalytic hydrogenation over Pt, the latter compound for example yielding only a dihydroderivative, $C_{18}H_{26}O_3$, $\lambda_{max}$ 236 m$\mu$ ($\epsilon$ 14,000), $\nu_{max}$ 1768 ($\gamma$-lactone), 1695 and 1627 cm$^{-1}$ (conjugated ketone), by reduction of the vinyl group. However, reduction of the hemiketal with lithium in liquid ammonia, followed by oxidation with chromium trioxide, gave material free from intense UV absorption, which contained a saturated five-ring carbonyl, $\nu_{max}$ 1740 cm$^{-1}$, in addition to the expected $\gamma$-lactone, $\nu_{max}$ 1776 cm.$^{-1}$ The retro-Michael reaction had therefore proceeded without fission of the five-membered ring to yield a cyclopentanone; furthermore, the conjugated double bond produced must be tetra-substituted since PMR spectra of compounds containing it showed only the olefinic protons of the vinyl group. Only two partial structures XIX and XX are possible for the cyclopentenone.



XIX



XX

Whilst cyclopentenones with an exocyclic double bond obey Woodward's rules for calculation of enone absorption, endocyclic compounds are exceptions, absorbing about 13 m$\mu$ lower than the calculated values.[31] The partial structure XIX would be

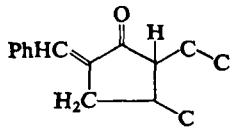
[29] T. Schmidlin and A. Wettstein, *Helv. Chim. Acta* **42**, 2636 (1959).

[30] Cf. R. E. Marker, R. B. Wagner, P. R. Ulshafer, E. L. Wittbecker, D. P. J. Goldsmith and C. H. Ruof, *J. Amer. Chem. Soc.* **69**, 2167 (1947).

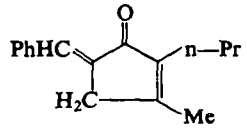
[31] Ref. 19, p. 55.

expected[31,32] to absorb above 250 mμ, clearly incompatible with the observed max at 236 mμ which is precisely that expected[31,33] for the alternative endocyclic system XX. Additional evidence, excluding the exocyclic system XIX comes from ozonolysis of the borohydride-reduced hemiketal, which afforded neutral material devoid of five-ring CO absorption in the IR.

Since alkaline treatment of an endocyclic cyclopentenone could induce migration of the double bond around the ring, it was necessary to confirm that the system XX was the direct product of a retro-Michael reaction involving the methine hydrogen of the cyclopentanone VI. The monobenzylidene derivative of hydroxy-A-dione, $C_{27}H_{34}O_3$, for which partial structure XXI follows from spectral data, $\lambda_{max}$ 295 mμ (ε 21,000),[34] $\nu_{max}$ 1710, 1696 (carbonyls), and 1630 $cm^{-1}$ (olefinic), in conjunction with the known environments VI and XI of the cyclopentanone and oxygen-B, on treatment with hot alkali rearranged to an amorphous product whose UV spectrum, $\lambda_{max}$ 307 mμ (ε 7000), was very similar to that[35] of the cross conjugated ketone XXII, $\lambda_{max}$ 309 mμ (ε 6300). A similar retro-Michael reaction is clearly occurring with both hydroxy-A-dione and its benzylidene derivative, and involves not the methylene but the methine hydrogen of the cyclopentanone VI.




XXI

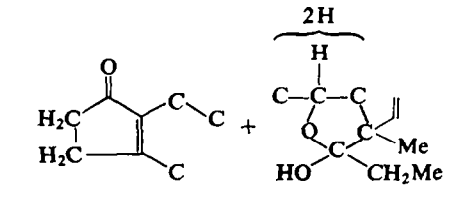



XXII

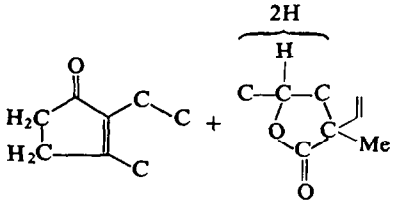
The presence of the intact cyclopentenone ring XX establishes that the hemiketal XVI and derived γ-lactone XVII arise from interaction of the OH function A and the carbonyl-B, and necessitates a 1,4-relationship between these oxygen functions. In conjunction with the previously-defined environments VI, XI and XIV, this leads to the partial structures XXIII and XXIV respectively for the hemiketal from the retro-Michael reaction and for the γ-lactone. The PMR spectrum of the latter compound XXIV showed the –COOC*H*– proton as a sharp quartet (1:1:1:1, $J_{AX}$ = 11·2 c/s, $J_{AY}$ = 6·2 c/s) at τ 4·93 resolvable from the vinyl proton absorption, which confirms the presence, suspected earlier in XIV, of only two neighbouring protons. The original hydroxy-A-dione must then contain the system XXV, opening as shown to the isomer XXVI, which then cyclizes to the hemiketal XXIII.

Treatment of mutilin trione, which must contain the structure XXVII, with hot methanolic alkali gave two products separable by chromatography. The less polar, $C_{20}H_{28}O_3$, was isomeric with the trione and had $\lambda_{max}$ 236 mμ (ε 13,200), $\nu_{max}$ 1699 (broad, carbonyls) and 1638 $cm^{-1}$ (olefinic) with no cyclopentanone absorption. In view of the stability of mutilin hydroxy-B-dione towards alkali, and the similarity of these spectral data with those of the cyclopentenone XXIII, this product is clearly the trione XXVIII, the direct analogue of XXIII, retro-Michael fission again being due to carbonyl-B.

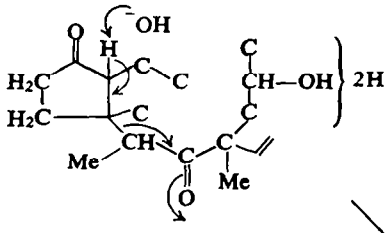
[32] H. S. French and L. Wiley, *J. Amer. Chem. Soc.* **71**, 3702 (1949).
[33] E. A. Brande and J. A. Coles, *J. Chem. Soc.* 1430 (1952).
[34] Ref. 19, p. 107.
[35] H. S. French, *J. Amer. Chem. Soc.* 74, 514 (1952).



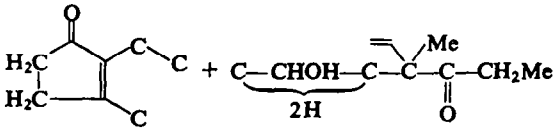
XXIII



XXIV

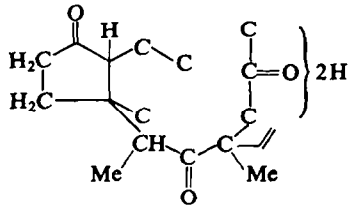


XXV

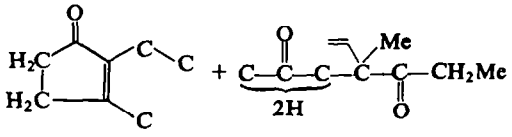


XXVI

Confirmation of this system XXVIII was provided by controlled Kuhn-Roth oxidation[28] to propionic and acetic acids, and by PMR spectroscopy which showed two tertiary Me groups (s, $\tau$ 8·52 and 8·71), one secondary (d, $\tau$ 9·04, $J=6$ c/s), and one primary (t, $\tau$ 9·01, $J=7{\cdot}5$ c/s), in addition to the vinyl protons at low field. Particularly

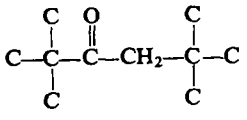


XXVII

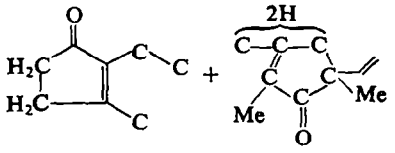


XXVIII

noticeable was the appearance for the first time of a sharp two proton singlet at $\tau$ 6·98. This can only be due to the two protons known to be adjacent to carbonyl-A, which must be present as methylene hydrogens in the system XXIX and become identical when the ring to which they are attached is opened.



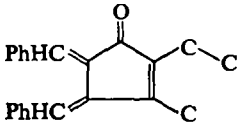
XXIX



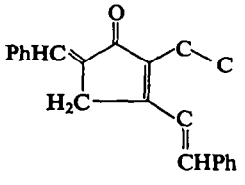
XXX

Further reaction of this trione XXVIII with alkali gave a compound, $C_{20}H_{26}O_2$, $\nu_{max}$ 1696 (carbonyls), 1637 and 1616 $cm^{-1}$ (olefinic), which was identical with the more polar, major product from alkaline treatment of mutilin trione itself. The intensity ($\epsilon$ 24,000) of the UV max at 243 m$\mu$ now indicated the presence of two conjugated enone chromophores, whilst propionic acid was no longer obtained on Kuhn-Roth oxidation. The compound clearly contains the system XXX, arising from XXVIII by aldol condensation and dehydration. PMR spectroscopy confirmed the presence, in addition to the vinyl group, of two tertiary C-Me groups (s, $\tau$ 8·51 and 8·76) and one secondary (d, $\tau$ 9·08, $J$=6·0 c/s). The allylic Me protons resonated at $\tau$ 8·42, as a triplet (1:2:1, $J$=2·0 c/s) split by long-range coupling[36] with the two methylene protons known (cf. XXIX) to be in the homo-allylic position.

Catalytic hydrogenation of this aldol product XXX reduced only the vinyl group, affording a dihydro derivative, $C_{20}H_{28}O_2$, $\nu_{max}$ 1696 (carbonyls), 1642 and 1623 $cm^{-1}$ (olefinic), with unaltered UV absorption, $\lambda_{max}$ 243 m$\mu$ ($\epsilon$ 23,500). However, reduction with lithium in liquid ammonia gave material transparent in the UV region, which on chromium trioxide oxidation showed, as expected, only cyclopentanone CO absorption, $\nu_{max}$ 1738 $cm^{-1}$. The dibenzylidene derivative, $C_{34}H_{34}O_2$, $\lambda_{max}$ 244, 268, and 355 m$\mu$ ($\epsilon$ 27,000, 22,000, and 27,300), obtained from XXX by reaction with benzaldehyde, contained the same extended UV chromophore (superimposed upon absorption due to a conjugated cyclopentenone) as the product, $\lambda_{max}$ 244, 271, and 335 m$\mu$ ($\epsilon$ 15,500, 17,500, and 25,500), from similar treatment of the hemiketal XXIII. Both derivatives must contain either the chromophore XXXI or XXXII, derived from the cyclopentenone system common to XXX and XXIII.



XXXI



XXXII

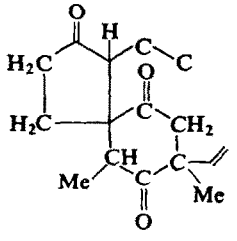
These facts are in full agreement with the partial formula XXVII for mutilin trione, which was deduced from that of the hydroxy-A-dione XXV.

The relation between the cyclopentanone CO and carbonyl-A was established by oxygenation of mutilin trione XXVII in the presence of potassium t-butoxide,[18] when one mole of oxygen was rapidly consumed, followed by alkaline hydrolysis. The resulting diketodicarboxylic acid, $C_{20}H_{30}O_6$, $\nu_{max}$ (in Nujol) 3400–2500 (carboxyls), 1705 (carboxyls and carbonyls), and 1636 $cm^{-1}$ (vinyl), $\lambda_{max}$ 300 m$\mu$ ($\epsilon$ 145), lacked the

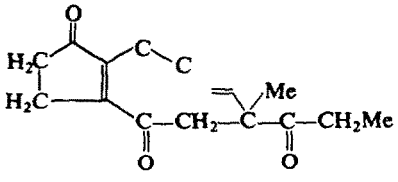
[36] N. S. Bhacca and D. H. Williams, *Applications of NMR Spectroscopy in Organic Chemistry* p. 110. Holden-Day, San Francisco (1964).

cyclopentanone system and contained one mole of water more than the triketomonocarboxylic acid, $C_{20}H_{28}O_5$, which would be the immediate oxidation product. The latter must therefore contain a 1,3-dicarbonyl system, necessarily generated in the oxidation since it cannot be present in XXVII (cf. carbonyl-A environment XXIX), which undergoes subsequent hydrolysis.

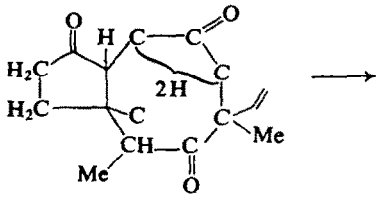
Clearly the cyclopentanone system is suffering the initial oxidative cleavage, as in IV to V; earlier work demonstrated its high reactivity under these conditions, whilst carbonyl-B was relatively inert, and the recently established environment (XXIX) of carbonyl-A precludes its fission to a ketocarboxylic acid. The new carbonyl thus formed can be in 1,3-relationship only to carbonyl-A, not to carbonyl-B (cf. XXVII). Thus the original cyclopentanone and carbonyl-A (XXIX) are 1,4-related, permitting the development of XXVII into two possible partial structures XXXIII and XXXIV for mutilin trione. Amongst considerable evidence against the former XXXIII in the work already described is the fact that the initial retro-Michael product from mutilin trione would contain the enedione system XXXV, for which there is neither spectroscopic evidence (cf. ref.[37]) nor is the product reducible by zinc in refluxing acetic acid. The alternative XXXIV for mutilin trione therefore stands.



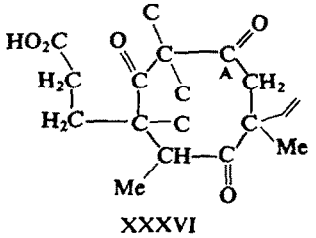
XXXIII



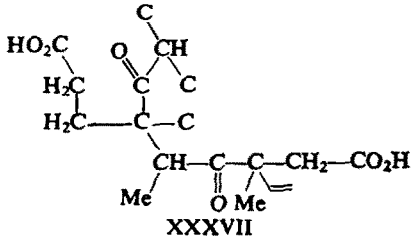
XXXV



XXXIV



XXXVI

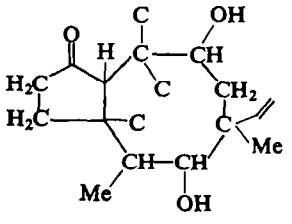


XXXVII

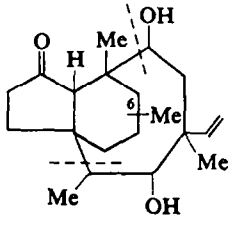
The immediate oxidation product of the trione XXXIV is then the 1,3-dione XXXVI, which is hydrolysed by base attack at carbonyl-A to the diketodicarboxylic acid XXXVII. Fission of the 1,3-dione by the alternative attack at the newly-generated

[37] Ref. 19, p. 61.

carbonyl group would lead to a substituted glutaric acid structure for the diketodicarboxylic acid, which is excluded since no anhydride is formed on heating. The ease of alkaline hydrolysis of the 1,3-dione XXXVI suggests that it is non-enolizable, whilst the fact that the diketodicarboxylic acid XXXVII gives a negative iodoform test necessitates the presence of at least one substituent on the central carbon of the 1,3-dione system. This permits us to orientate as in XXXVI and XXXVII the environment XXIX established for carbonyl-A, which when applied to the trione XXXIV leads unequivocally to the medium-ring system XXXVIII for mutilin itself.



XXXVIII



XXXIX

*The structures of mutilin and pleuromutilin*

Since mutilin contains a third carbocyclic ring, together with one more secondary and one more tertiary C-Me group than are accounted for in XXXVIII, but no higher C-alkyl groups, only two types of complete structure are possible. One, involving a spiro-methylcyclobutane system adjacent to hydroxy-A, can be immediately rejected since it offers no satisfactory explanation of the mass spectroscopic and dehydrogenation evidence, and as a diterpene is biogenetically very implausible. The only alternative structure for mutilin is the hydroindanone system XXXIX. In this case, electron impact fragmentation of mutilin derivatives occurs predictably by cleavage of the medium-ring bonds indicated in XXXIX, together with hydrogen transfer, to give trihydroindane-type ions as the observed base peaks of the mass spectra. The formation of a second six-membered ring during dehydrogenation of the corresponding dihydrotriol triacetate to alkylfluorenes may well involve an intramolecular Diels-Alder addition, in a poly-unsaturated intermediate, of the medium-ring chain to the five-membered ring.

We have no chemical evidence defining the position of the secondary Me group on the cyclohexane ring, but tracer evidence[6] (see later), confirming the biosynthetic pathway suggested by Dr. Arigoni,[7-9] indicates $C_6$ as the most probable site.

Pleuromutilin itself is then the glycollic ester I of the OH function A of mutilin XXXIX.

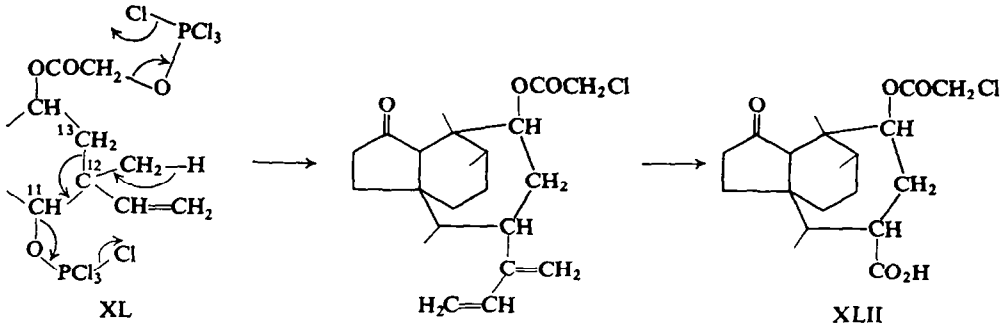
*Further reactions of the medium ring*

A number of reactions involving the medium ring of pleuromutilin and its derivatives were observed which are of interest, and which provide further support for the structure I since they can be readily rationalized on this basis.

(i) *Contraction.* Treatment of pleuromutilin I with phosphorus pentachloride resulted in chlorination of the primary glycollic ester OH and elimination with rearrangement, as in XL, of the secondary hydroxyl-B, yielding the dienechloroacetate XLI, $C_{22}H_{31}ClO_3$, $\nu_{max}$ 1747 (chloroacetate), 1732 (cyclopentanone), 3065 and 1635 (vinyl and vinylidene), 922 (vinyl[38]), and 895 $cm^{-1}$ (vinylidene[38]). The PMR spectrum

[38] Ref. 17, p. 34.

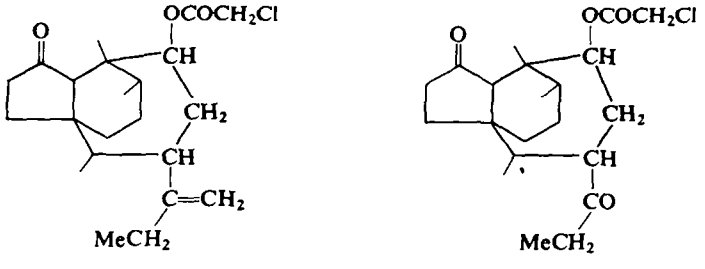
of the product XLI indicated loss of a tertiary C-Me group, and showed resonances due to the remaining tertiary Me (s, $\tau$ 8·58), the two secondary Me's (superimposed



XL XLI XLII

d, $J \approx 6$ c/s, $\tau$ 9·25) and the chloroacetyl methylene (s, $\tau$ 6·00), together with a six-proton complex at low field containing the methine proton $-CH(OCOCH_2Cl)-$ and the five olefinic protons. The butadiene system, giving rise to an UV max at 226 m$\mu$ ($\epsilon$ 16,200),[39] was established structurally by ozonolysis to formaldehyde (1·4 moles) and (after oxidation with hydrogen peroxide) the anticipated trisnor-carboxylic acid XLII, $C_{19}H_{27}ClO_5$, $\nu_{max}$ 3500–2500 and 1709 (carboxyl), and 1740 cm$^{-1}$ (chloroacetate and cyclopentanone).

Dihydropleuromutilin afforded similarly the monoenechloroacetate XLIII, $C_{22}H_{33}ClO_3$, $\nu_{max}$ (in $CS_2$) 1745 (chloroacetate), 1733 (cyclopentanone), 3050, 1633, and 892 cm$^{-1}$ (vinylidene[38]), which on ozonolysis gave formaldehyde (0·6 mole) and the norketone XLIV, $C_{21}H_{31}ClO_4$, $\nu_{max}$ (in Nujol) 1738, 1730 (chloroacetate, cyclopentanone), and 1702 cm$^{-1}$ (ketone). Confirmation of these structures XLIII and XLIV was provided by the PMR spectrum of the latter, which showed a primary Me triplet ($J = 7·2$ c/s, $\tau$ 8·91), in addition to resonances due to a tertiary Me (s, $\tau$ 8·58), two secondary Me's (overlapping d, $J \approx 5$ c/s, $\tau$ 9·22), and the methine (1:1:1:1 quartet, $J_{AX} = 11$ c/s, $J_{AY} = 3·5$ c/s, $\tau$ 4·47) and chloroacetyl methylene (s, $\tau$ 5·98) protons of the $-CH_2CH(OCOCH_2Cl)-$ system.



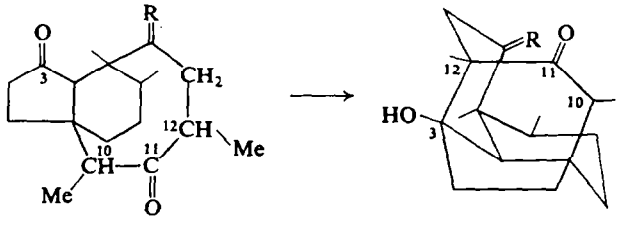
XLIII XLIV

This ready contraction of the medium ring of pleuromutilin derivatives suggests that if the phosphorus pentachloride rearrangement is of the usual type,[40] then the four centres (oxygen, $C_{11}$, $C_{12}$, and $C_{13}$ in XL) can assume a coplanar conformation.

[39] Ref. 19, p. 45.

[40] D. H. R. Barton, *J. Chem. Soc.* 1027 (1953).

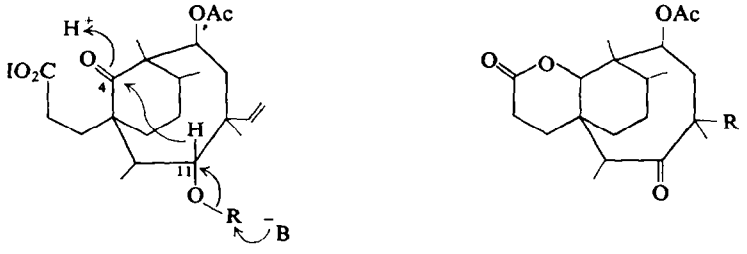
(ii) *Intramolecular aldol cyclization.* The bisnor-hydroxy-A-dione XLV (R = H, OH), obtained as described earlier by removal of the vinyl group from mutilin hydroxy-A-dione, was converted by alkali into an isomeric dihydroxymonoketone, $C_{18}H_{28}O_3$, $\nu_{max}$(in Nujol) 3385, 3307 (hydroxyls), and 1706 $cm^{-1}$ (carbonyl), with loss of the cyclopentanone CO. Whereas the starting dione contained only one tertiary Me, the PMR spectrum (in pyridine containing $D_2O$) of the product showed two such groups (s, $\tau$ 8·23 and 8·60), in addition to a quartet (1:1:1:1, $J_{AX}$ = 12 c/s, $J_{AY}$ = 6·2 c/s) at $\tau$ 5·62 due to the methine proton of the hydroxyl-A system $-CH_2CHOD-$. These facts indicate formation of the tetracyclic aldol XLVI (R = H, OH) by a ring-closure which must involve $C_{12}$, not $C_{10}$, since it is observed only when a carbanion can be generated at that position.



XLV XLVI

The presence of the tertiary OH in the aldol XLVI (R = H, OH) was confirmed when oxidation with chromium trioxide gave an hydroxydione $C_{18}H_{26}O_3$, XLVI (R = O), $\nu_{max}$ 3590 (hydroxyl) and 1706 $cm^{-1}$ (carbonyls). The identical hydroxydione was formed, undoubtedly via the intermediate decarboxylated bisnor-trione XLV (R = O), by alkali treatment of the norcarboxylic acid, $C_{19}H_{26}O_5$, obtained from ozonolysis of mutilin trione.

(iii) *Transannular hydride migrations.* Mild treatment with potassium t-butoxide in t-butanol of the diacetoxyketocarboxylic acid XLVII (R = Ac; partial structure V) obtained by oxygenation of diacetylmutilin afforded not the hydroxy-acid XLVII (R = H) expected by cleavage of the more reactive acetate on hydroxyl-B, but an acetoxyketo-δ-lactone $C_{22}H_{32}O_5$, XLVIII (R = CH=$CH_2$), $\nu_{max}$ 3075, 1631 and 927 (vinyl), 1757 (δ-lactone[41]), 1740 (acetate), and 1700 $cm^{-1}$ (ketone). A PMR singlet at $\tau$ 6·34 can be ascribed to the isolated proton at the δ-lactone terminus, the resonance position indicating considerable shielding probably from the medium ring, whilst the one-proton quartet at $\tau$ 6·82 (1:3:3:1, $J$ = 6·5 c/s) is indicative of the carbonyl-B system $-CH$MeCO– (see above). The PMR spectrum showed also the vinyl pattern, two



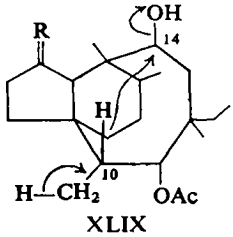
XLVII XLVIII

[41] Ref. 17, p. 178.

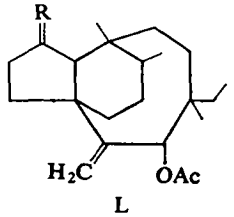
tertiary and two secondary Me's, and the Me singlet ($\tau$ 7·93) and broadened methine doublet ($\tau$ 4·10, $J$=9 c/s) of the acetylated hydroxyl-A region $-CH_2CH(OCOCH_3)-$. Chemical proof of structure XLVIII (R=CH=$CH_2$) followed from its ozonolysis and subsequent oxidation with hydrogen peroxide to an acid XLVIII (R=$CO_2H$) which on warming readily decarboxylated to the bisnor-lactone XLVIII (R=H), $C_{20}H_{30}O_5$, $\nu_{max}$ 1756 (δ-lactone[41]), 1740 (acetate), and 1704 $cm^{-1}$ (ketone).

Alkaline treatment of the acid XLVII (R=Ac) promotes a transannular 1,4-hydride migration, a phenomenon familiar in medium-ring chemistry,[42] from $C_{11}$ to $C_4$ as shown. Mechanistically the reaction is similar to the Meerwein-Ponndorf reduction, but is assisted here by the spatial proximity of the hydride donor and acceptor.

The dihydromutilin mono-acetate XLIX (R=O) in which hydroxyl-A is free, was converted smoothly by phosphorus oxychloride in pyridine at room temperature into an anhydro derivative, $C_{22}H_{32}O_3$, $\nu_{max}$ (in $CS_2$) 1732 (cyclopentanone and acetate), 3060 and 1627 $cm^{-1}$ (olefinic). The low field region of the PMR spectrum of this product showed incompletely resolved absorption equivalent to two olefinic protons plus the expected methine proton of the acetylated hydroxyl-B, as a sharp resonance (0·5 H) at $\tau$ 4·79 and a somewhat broader resonance (2·5 H) at $\tau$ 4·72. At higher field were visible, apart from the acetoxyl peak at $\tau$ 7·96, only four C-Me resonances, resolvable into two tertiary Me singlets at $\tau$ 8·78 and 9·08, a secondary Me doublet ($J$=5·5 c/s) at $\tau$ 9·26, and a primary Me triplet ($J$=7·5 c/s) at $\tau$ 9·19. A secondary Me has been lost in the reaction, which cannot therefore be a simple elimination to yield the observed disubstituted olefin. The product probably has the exocyclic methylene structure L (R=O), arising by 1,5-hydride migration from $C_{10}$ to $C_{14}$ as shown in XLIX, by analogy with the similar reaction reported by Dr. Arigoni[8] for the desoxy compound XLIX (R=$H_2$). The failure of the exocyclic methylene group to give any formaldehyde on ozonolysis may be due to extreme steric hindrance.



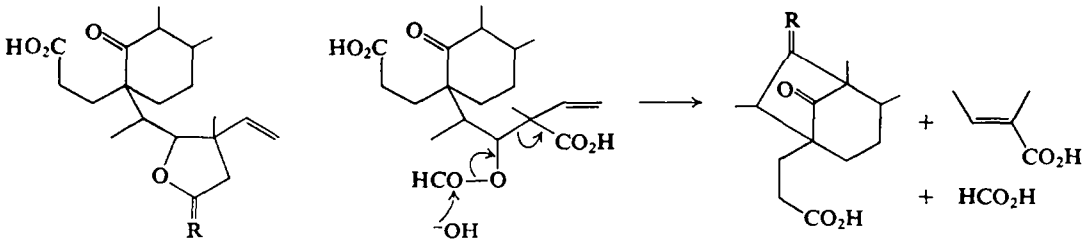

XLIX




L

(iv) *Retro-aldol fission.* The diacetoxyketocarboxylic acid XLVII (R=Ac) when heated with aqueous base did not yield the corresponding dihydroxyketo-acid. Instead, this intermediate suffered retro-aldol cleavage of the medium ring, the resulting γ-hydroxy-aldehyde cyclizing to the hemi-acetal LI (R=H, OH), $C_{20}H_{32}O_5$, $\nu_{max}$ 3500–2600 (carboxyl), 3370 (hydroxyl), 1704 (carboxyl and ketone), and 1638 $cm^{-1}$ (vinyl). The oxidation with chromium trioxide of this keto-acid hemi-acetal LI (R=H, OH) as its Me ester gave a neutral fraction, which on saponification afforded the keto-acid γ-lactone LI (R=O), $C_{20}H_{30}O_5$, $\nu_{max}$ 3500–2600 (carboxyl), 1782 (γ-lactone), 1702 (ketone), 1639 and 922 $cm^{-1}$ (vinyl). As expected, this same γ-lactone resulted from alkaline oxygenation of mutilin acetoxy-B-dione, followed by hydrolytic fission of the medium-ring 1,3-dione thus generated, deacetylation and lactonization.

[42] A. C. Cope, M. M. Martin, and M. A. McKervey, *Quart. Rev. Chem. Soc.* **20**, 119 (1966).



LI LII LIII

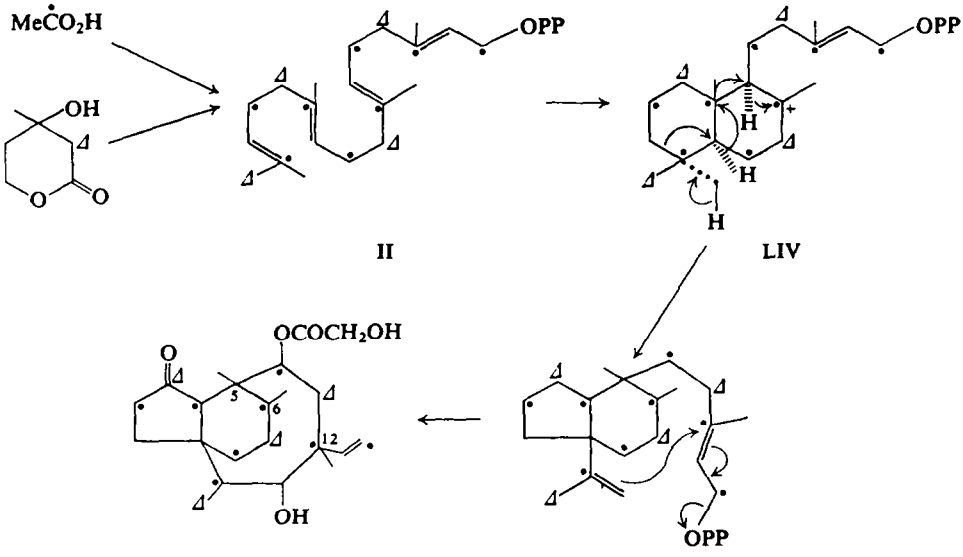
Oxidation with chromium trioxide of the keto-acid hemi-acetal LI (R=H, OH) itself gave a dicarboxylic acid, $C_{20}H_{30}O_7$, which was also formed on mild hydrolysis of the acidic fraction from the above oxidation of the Me ester. The dicarboxylic acid is probably LII, in agreement with IR max at 3500–2600 (carboxyls), 1740 (formate), 1702 (carboxyls and ketone), and 1634 $cm^{-1}$ (vinyl), and arises by oxidative fission of the enol ether bond of the dehydrated hemi-acetal.[30] In support, base-catalysed hydrolysis of the formate LII and retroaldolization of the resulting $\beta$-hydroxy-acid afforded tiglic acid, together with formic acid and an acid $C_{14}H_{22}O_4$. The IR spectrum of this $C_{14}$-acid, $\nu_{max}$ (in Nujol) 3500–2500, 3250, and 1730 $cm^{-1}$, indicated the presence of OH and 5-ring ketone groupings in addition to the carboxyl, and it is formulated as the internal aldol product LIII (R=H, OH) of the initially-formed aldehydo-ketocarboxylic acid. Oxidation gave the corresponding diketocarboxylic acid LIII (R=O), $C_{14}H_{20}O_4$, $\nu_{max}$ 1727 (ketones) and 1713 $cm^{-1}$ (carboxyl). This was confirmed as the expected 1,3-dione by alkaline hydrolysis to a mixture of dicarboxylic acids, sublimation of which yielded a neutral fraction showing IR max at 1806 and 1762 $cm^{-1}$ characteristic of a glutaric anhydride,[43] in addition to ketonic absorption at 1705 $cm^{-1}$.

*Probable biosynthetic pathway*

Dr. Arigoni[7–9] proposed for pleuromutilin the probable biosynthetic pathway (II→LIV→LV→I), and evidence as to its general validity has already been presented.[6–8] This route locates at $C_6$ the secondary Me group on the cyclohexane ring unplaced by our chemical work, and additional evidence in its support was obtained by degradation of biogenetically-labelled material.

[1-$^{14}$C]Acetate-derived mutilin (r.m.a.[11] $\times 10^{-4}$, 41·6) and the corresponding trione (which would be unlikely to undergo Wagner-Meerwein rearrangement) on Kuhn-Roth oxidation gave acetic acid (2·02, 2·45 moles respectively) which was degraded by the Schmidt procedure. The acid was unlabelled in its Me group but carried in its carboxyl group (r.m.a. $\times 10^{-4}$ as barium carbonate, 3·94 and 3·88 respectively) three-quarters of one-eighth of the total $C_{20}$-activity. In the precursor II all the Me groups are attached to carbons derived from acetate carboxyl, which would each carry one-eighth of the total activity. Therefore, as in the analogous case of rosenonolactone,[10] one of the four Me groups of mutilin has migrated during the cyclization from its original labelled position in II to an inactive carbon derived from acetate Me. The observed dilution of activity is clearly caused by the migrated Me at $C_5$.

[43] Ref. 17, p. 127.




Mutilin samples labelled by [1-$^{14}$C]acetic acid and [2-$^{14}$C]mevalonic lactone (r.m.a. $\times 10^{-4}$, 41·6 and 59·1 respectively) were degraded to the diacetoxyketocarboxylic acid XLVII (R = Ac), from which carbon dioxide (r.m.a. $\times 10^{-4}$, 0·24 and 14·1 respectively) was released by Schmidt decarboxylation. As anticipated, the cyclopentanone carbonyl is not derived from acetate carboxyl, but carries one-quarter of the molar activity of mutilin prepared from [2-$^{14}$C]mevalonate.

Formaldehyde, representing the Me carbon at $C_{12}$, isolated from ozonolysis of the ring-contracted monoenechloroacetate (XLIII) was inactive regardless of whether [1-$^{14}$C]acetate- or [2-$^{14}$C]mevalonate-labelled material was used. This provides further support for the structure XLIII.

*Stereochemistry*

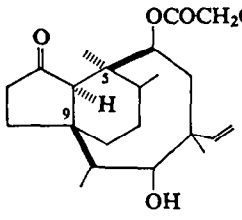
Direct experimental work on the stereochemistry of pleuromutilin was abandoned in view of the work of Dr. Arigoni in progress, but some observations may be made from our own results.

Considering the three asymmetric centres at the ring junctions, there are 8 possible stereochemical arrangements corresponding to 4 mirror-image pairs. Models show that two of these pairs, in which the medium-ring linkages to the hydroindanone nucleus at $C_5$ and $C_9$ are *trans* related, are highly strained. Moreover, the facile ring-contraction induced by phosphorus pentachloride would be impossible in these cases, which therefore need not be considered further. The two remaining pairs, which have the medium ring *cis*-linked to the nucleus and differ in the configuration of the methine hydrogen at $C_4$, are quite flexible.

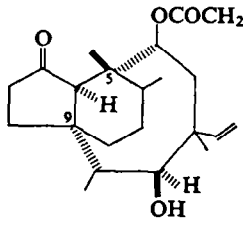
The ORD curve* of pleuromutilin shows a positive Cotton effect (ORD in MeOH $[\Phi]_{600} -180°$, $[\Phi]_{400} +370°$, $[\Phi]_{320} +3200°$, $[\Phi]_{278} -3600°$). The curves of alicyclic monoketones usually reflect only the immediate environment of the CO, although in the present case conformational effects due to the medium ring could interfere. In simple

*We are indebted to Professor W. Klyne for ORD measurements.

hexahydroindanones[44] the skewed nature of the five-membered ring itself exerts a strong influence on the symmetry of the CO group, and neither the nature of angular substituents nor other non-chromophoric substituents play an important part. Comparison of the ORD curve of pleuromutilin with those of other hexahydroindanones,[44,45] assuming no medium-ring interference, restricts the absolute stereochemistry at the ring-junctions to LVI or LVII.




LVI




LVII

Mutilin is not isomerized by acid or base, and its ORD curve is closely similar to that of the parent pleuromutilin. The indanone system would permit equilibrium of the centre adjacent to the CO, but this is clearly in the same stable form in both compounds. It is not, however, possible in this complex case to assume that stability indicates the *cis* cyclohexane–cyclopentanone junction which would prevail in simple systems.[46] If this junction is *cis* (as in LVII) in pleuromutilin and mutilin, it must invert during formation of the aldol XLVI, since only the *trans* will enable this cyclization to occur; there is no inherent difficulty about this inversion to give a final stable product.

Of these two possible steric arrangements LVI and LVII, the latter is in fact that which would result if the biosynthesis of pleuromutilin proceeded through an intermediate LIV carrying the normal absolute diterpenoid stereochemistry, as is the case in rosenonolactone biosynthesis.[10,47] The arrangement LVI would be derived from an intermediate of antipodal stereochemistry to LIV, such as is involved[10,47] in gibberellic acid biosynthesis, followed by epimerization adjacent to the carbonyl group. Assuming LVII to be the more likely of the two, then the steric requirements of the internal Meerwein-Ponndorf reaction (XLVII: R = Ac→XLVIII) described above are best met if the $C_{11}$-hydroxyl has the $\beta$-orientation shown in LVII. It is also notable that with this configuration at $C_{11}$, the medium ring readily adopts a conformation which would facilitate the ring contractions (XL→XLI, and its dihydro-analogue) by virtue of coplanarity of the four centres involved.

## EXPERIMENTAL

*General.* M. ps determined on a Kofler stage are uncorrected. UV and IR spectra were measured in EtOH and $CCl_4$ solns respectively, except where otherwise stated. Light petroleum refers to the fraction b.p. 60–80°. Alumina used was Peter Spence Grade "H"; Florisil was supplied by the Floridin Co., Warren, Pennsylvania. Radioactivity was assayed as previously described.[11] PMR spectra were recorded on a Varian Associates A60 machine for ca. 10% solns in $CDCl_3$ containing TMS as internal reference, except where otherwise specified.

[44] W. Klyne, *Tetrahedron* **13**, 29 (1961).

[45] C. Djerassi, *Optical Rotatory Dispersion* pp. 41, 73 and 89. McGraw-Hill, New York (1960).

[46] Cf. L. F. Fieser and M. Fieser, *Steroids* p. 212. Reinhold, New York (1959).

[47] Cf. R. McCrindle and K. H. Overton, *Advances in Organic Chemistry; Methods and Results* **5**, p. 47, Interscience, New York (1965), and Refs cited therein.

*Pleuromutilin*

*Pleurotus mutilus* (Fr.) Sacc. was cultured as described.[1] After 28 days' growth the medium was extracted with chf and the pleuromutilin (100–250 mg/l. of medium), m.p. 170–171° (lit.[3] 170–171°), $\nu_{max}$ (in $CS_2$) 3550, 1736, and 917 $cm^{-1}$ isolated by chromatography on Florisil in benzene-ether (4:1) and crystallization from AcOEt–light petroleum. The bis-3,5-dinitrobenzoate had m.p. 245–247° (lit.[3] 249–250°).

After 19 days' growth, sodium [1-$^{14}$C]acetate (0·3 mC) was distributed among 6 flasks each containing 750 ml of medium. The radioactive metabolite (1·1 g), isolated as above, was diluted with inactive pleuromutilin (0·9 g) and purified. (Found: r.m.a. × $10^{-4}$, 44·3). [2-$^{14}$C]Mevalonic lactone (0·1 mC) similarly afforded labelled pleuromutilin (650 mg). (Found: r.m.a. × $10^{-4}$, 58·6).

Hydrogenation of pleuromutilin (250 mg) in AcOEt (40 ml) over Pd-C (250 mg, 10%) gave dihydropleuromutilin, m.p. 147–148° from AcOEt (lit.[3] 149–150°), $\nu_{max}$ (in Nujol) 3570, 3420, 1730, and 1724 $cm^{-1}$.

Pleuromutilin (160 mg) in $Ac_2O$–pyridine (1:2, 4 ml) was stood for 10 hr at room temp. Chromatography of the product on alumina and elution with benzene furnished pleuromutilin diacetate (47 mg), m.p. 144–145° from light petroleum (lit.[3] 145·5°), $\nu_{max}$ 1750–1725 and 919 $cm^{-1}$. Further elution with benzene–ether (10:1) gave *pleuromutilin monoacetate* (83 mg), m.p. 120–121° from ether–light petroleum $\nu_{max}$ 3580 and 1739 cm.$^{-1}$ (Found: C, 68·7; H, 8·6. $C_{24}H_{36}O_6$ requires: 68·6; H, 8·7%).

*Mutilin*

Pleuromutilin (800 mg) was saponified[3] to yield mutilin (700 mg), m.p. 191·5–193·5° (lit.[3] 193–194° from aqueous EtOH, $\nu_{max}$ 3600, 3065, 1735, 1635, and 905 $cm^{-1}$. (Found: r.m.a. × $10^{-4}$, 41·6 and 59·1 from [1-$^{14}$C]acetate- and [2-$^{14}$C]mevalonate-labelled pleuromutilin respectively). The saponification mother liquors were acidified, evaporated to dryness, and the residue extracted with ether to afford glycollic acid (135 mg, 84%), m.p. 75–79° from ether, identified by mixed m.p., IR spectrum and $R_F$ value (0·80) on paper chromatography[48] in comparison with authentic material. (Found: r.m.a. × $10^{-4}$, 4·67 from [1-$^{14}$C]acetate-derived pleuromutilin).

Mutilin was recovered (85%) after heating under reflux with 7N methanolic KOH under N for 25 min.

Paper chromatography[49] of the volatile acids produced on controlled oxidation of mutilin with $CrO_3$[28] showed the presence of AcOH ($R_F$ 0·14) only. Kuhn-Roth oxidation of [1-$^{14}$C]acetate-derived mutilin gave AcOH (2·02 moles) degraded by the Schmidt procedure[50] to $CO_2$ and methylamine which were assayed as $BaCO_3$ and 2,4-dinitro-N-methylaniline. (Found: r.m.a. × $10^{-4}$, 3·94 and 0 respectively).

Mutilin (50 mg) and hydroxylamine hydrochloride (50 mg) in pyridine (1 ml) were heated on the steam-bath for 3 hr. Crystallization of the product from benzene gave *mutilin oxime*, m.p. 231–233°. (Found: C, 71·9; H, 9·7; N, 4·2. $C_{20}H_{33}O_3N$ requires: C, 71·6; H, 9·9; N, 4·2%).

Mutilin (50 mg) in AcOEt (8 ml) was hydrogenated over Pd-C (50 mg, 10%), yielding *dihydromutilin* m.p. 223–224° from benzene–light petroleum. (Found: C, 74·3; H, 10·7. $C_{20}H_{34}O_3$ requires: C, 74·5; H, 10·7%). The compound was recovered after treatment for 30 min with $O_3$ in AcOH.

Mutilin (800 mg) in $Ac_2O$–pyridine (1:2, 20 ml) was stood at room temp for 22 hr and then warmed on the steam-bath for 30 min. Chromatography of the products on alumina gave, on elution with benzene, *mutilin diacetate* (12 mg), m.p. 210–211° from benzene–light petroleum, $\nu_{max}$ (in $CS_2$) 1730, 1723, and 915 $cm^{-1}$. (Found: C, 71·2; H, 9·0. $C_{24}H_{36}O_5$ requires: C, 71·3; H, 9·0%). Further elution with benzene–chf (10:1) furnished *mutilin monoacetate* (95 mg), m.p. 177–178° from aqueous EtOH, $\nu_{max}$ 3620 and 1737 $cm^{-1}$. (Found: C, 72·7; H, 9·4. $C_{22}H_{34}O_4$ requires: C, 72·9; H, 9·5%).

Mutilin with $Ac_2O$–pyridine at 100° for 5 hr gave the diacetate (>80%).

*Mutilin trione and its dihydro derivative*

Mutilin (164 mg) in acetone (30 ml) was treated dropwise with 0·8 N $CrO_3$ in 9 N $H_2SO_4$[51] (uptake 2·1 0). Dilution with water and extraction with ether gave *mutilin trione* (156 mg), m.p. 108–109°

[48] J. B. Stark, A. E. Goodban, and H. S. Owens, *Analyt. Chem.* **23**, 413 (1951).

[49] R. L. Reid and M. Lederer, *Biochem. J.* **50**, 60 (1951).

[50] E. F. Phares, *Arch. Biochem. Biophys.* **33**, 173 (1951).

[51] A. Bowers, T. G. Halsall, E. R. H. Jones, and A. J. Lemin, *J. Chem. Soc.* 2548 (1953).

from aqueous MeOH, $\nu_{max}$ 1740, 1702, and 926 cm$^{-1}$, $\lambda_{max}$ 303 m$\mu$ ($\epsilon$ 155). (Found: C, 76·0; H, 9·0. $C_{20}H_{28}O_3$ requires: C, 75·9; H, 8·9%).

As with mutilin itself, controlled chromic acid oxidation of mutilin trione gave AcOH as the only volatile acid, whilst Schmidt degradation of AcOH (2·45 moles) produced by Kuhn-Roth oxidation of the [1-$^{14}$C]acetate-derived trione afforded $BaCO_3$ and 2,4-dinitro-N-methylaniline. (Found: r.m.a. × 10$^{-4}$, 3·88 and 0 respectively).

Hydrogenation of the trione (150 mg) in AcOEt (15 ml) over Pd-C (80 mg, 10%) gave *dihydromutilin trione*, m.p. 113–114° from aqueous MeOH, $\nu_{max}$ (in $CS_2$) 1744, 1700, and 919 cm$^{-1}$. (Found: C, 75·7; H, 9·3. $C_{20}H_{30}O_3$ requires: C, 75·5; H, 9·5%).

*Ozonolysis of mutilin*

Mutilin (80 mg) in AcOH (10 ml) was ozonized to completion. Zn dust (200 mg) and water (5 ml) were added, and the mixture stirred at 60° for 1 hr. Steam distillation into 2,4-dinitrophenylhydrazine in 2N HCl gave formaldehyde 2,4-dinitrophenylhydrazone (36 mg), m.p. 163–165° from aqueous EtOH, identified by mixed m.p. and paper chromatography,[52] $R_F$ 0·18. (Found: r.m.a. × 10$^{-4}$, 5·16 from [1-$^{14}$C]acetate-derived mutilin). Neutralization of the residual liquors and extraction with ether afforded *nor-mutilin aldehyde* (53 mg), m.p. 214–217° from aqueous EtOH, $\nu_{max}$ (in Nujol) 3550, 3380, 1726, and 1700 cm$^{-1}$. (Found: C, 70·9; H, 9·2. $C_{19}H_{30}O_4$ requires: C, 70·8; H, 9·4%). (Found: r.m.a. × 10$^{-4}$, 36·1 from [1-$^{14}$C]acetate-derived mutilin).

In a similar experiment, the ozonide from mutilin (80 mg) was reacted with $H_2O_2$ (2 ml, 30 vols) for 3 hr at room temp. After destruction of the excess of peroxide with Pd-C, the product was separated into acidic (65 mg) and neutral (17 mg) fractions. Crystallization of the acidic fraction from EtOH–light petroleum gave *nor-mutilin carboxylic acid* m.p. 254–258° (dec), $\nu_{max}$ (in Nujol) 3615, 3317, 3500–2700, 1736, and 1708 cm$^{-1}$, $\lambda_{max}$ 295 m$\mu$ ($\epsilon$ 34). (Found: C, 67·2; H, 8·9. $C_{19}H_{30}O_5$ requires: C, 67·5; H, 9·0%).

*Nor-mutilin trione carboxylic acid*

Mutilin trione (80 mg) in AcOEt (10 ml) was ozonized to completion. The product after oxidative work-up by addition of $H_2O_2$ (2 ml, 100 vols) was separated into acidic (69 mg) and neutral (negligible) fractions. Crystallization of the former from $CCl_4$ gave *nor-mutilin trione carboxylic acid*, m.p. 154–157° (dec), $\nu_{max}$ 3400–2600, 1739, and 1706 cm$^{-1}$. (Found: C, 68·1; H, 7·7. $C_{19}H_{26}O_5$ requires: C, 68·3; H, 7·9%).

*Mutilin hydroxy-A-dione*

(i) *From pleuromutilin.* Pleuromutilin (82 mg) in acetone (20 ml) was reacted with 0·8N $CrO_3$ (2 mols) in 9N $H_2SO_4$ for 15 min at room temp. After destruction of the unused oxidant with isopropanol, the product was separated into neutral (59 mg) and acidic (20 mg) fractions. Crystallization of the neutral fraction from benzene–light petroleum gave the *diketoglycollate*, m.p. 183–184°, $\nu_{max}$ 3580, 1735, and 1702 cm$^{-1}$. (Found: C, 70·1; H, 8·6. $C_{22}H_{32}O_5$ requires: C, 70·2; H, 8·6%).

The diketoglycollate (45 mg) was hydrolysed in 1N methanolic KOH (2·5 ml) under reflux for 15 min, affording *mutilin hydroxy-A-dione* (23 mg), m.p. 162–163° from benzene–light petroleum, $\nu_{max}$ 3655, 1740, and 1702 cm$^{-1}$, $\lambda_{max}$ 303 m$\mu$ ($\epsilon$ 318). (Found: C, 75·4; H, 9·4. $C_{20}H_{30}O_3$ requires: C, 75·5; H, 9·5%).

Similar saponification of the acidic fraction from the oxidation gave the same hydroxy-A-dione (60%), together with oxalic acid identified by paper chromatography in two solvent systems, $R_F$ 0·33[53] and 0·12.[54]

(ii) *From mutilin.* Mutilin (54 mg) in acetone (10 ml) was oxidized with 0·8N $CrO_3$ (1 mol) in 9N $H_2SO_4$. Chromatography of the product on alumina and elution with benzene–ether (10:1) gave mutilin hydroxy-A-dione (34 mg, 63%), identified by direct comparison.

Acetylation of the hydroxy-A-dione with $Ac_2O$–pyridine on the steam-bath for 2 hr furnished *mutilin hydroxy-A-dione acetate*, m.p. 138–139° from aqueous MeOH, $\nu_{max}$ 1734 and 1698 cm$^{-1}$. (Found: C, 73·5; H, 9·0. $C_{22}H_{32}O_4$ requires: C, 73·3; H, 9·0%).

Mutilin hydroxy-A-dione was recovered after treatment with 2,4-dinitrophenylhydrazine in methanolic $H_2SO_4$ on the steam-bath for 30 min, or with PhMgBr in refluxing ether for 4 hr.

[52] L. Horner and W. Kirmse, *Liebigs Ann* **597**, 48 (1955).

[53] J. Opienska-Blauth, O. Saklawska-Szymonowa and M. Kanski, *Nature Lond.* **168**, 511 (1951).

[54] A. G. Long, J. R. Quayle and R. J. Stedman, *J. Chem. Soc.* 2197 (1951).

*Mutilin hydroxy-B-dione*

Mutilin monoacetate (107 mg) in acetone (25 ml) was oxidized by 0·8 N $CrO_3$ (1 mole) in 9 N $H_2SO_4$ to *mutilin hydroxy-B-dione acetate* (85 mg), crystals m.p. 207–208° from aqueous MeOH, $\nu_{max}$ 1735 and 1698 $cm^{-1}$, $\lambda_{max}$ 300 m$\mu$ ($\epsilon$ 54). (Found: C, 73·1; H, 9·0. $C_{22}H_{32}O_4$ requires: C, 73·3; H, 9·0%).

Hydrolysis of this acetate (50 mg) in 1 N methanolic KOH under reflux for 15 min gave, after crystallization from light petroleum, *mutilin hydroxy-B-dione*, m.p. 128–130°, $\nu_{max}$ 3570, 1737, and 1698 $cm^{-1}$. (Found: C, 75·5; H, 9·4. $C_{20}H_{30}O_3$ requires: C, 75·5; H, 9·5%).

Acetylation of the hydroxy-B-dione with $Ac_2O$–pyridine on the steam-bath for 1 hr afforded the parent acetate in high yield. The hydroxy-B-dione was recovered after warming with 2,4-dinitrophenylhydrazine in methanolic $H_2SO_4$ on the steam-bath for 30 min, and after heating under reflux with 7 N methanolic KOH under N for 25 min.

*Reduction of mutilin with* LAH

Mutilin (125 mg) in dry THF was added dropwise to a stirred suspension of LAH (50 mg) in the same solvent (50 ml). After refluxing for 2 hr, the excess of hydride was decomposed with AcOEt. Working up in the usual way gave *mutilin triol* (98 mg), m.p. 180–182° from benzene, $\nu_{max}$ (in Nujol) 3618, 3482, 3070, 1640, and 905 $cm^{-1}$. (Found: C, 74·3; H, 10·6. $C_{20}H_{34}O_3$ requires: C, 74·5; H, 10·7%).

Hydrogenation of this triol in AcOEt over 10% Pd–C afforded *mutilin α-dihydrotriol*, m.p. 198–199° from benzene, $\nu_{max}$ (in Nujol) 3460 $cm^{-1}$. (Found: C, 74·1; H, 11·3. $C_{20}H_{36}O_3$ requires: C, 74·0; H, 11·2%).

*Reduction of mutilin with lithium in ammonia*

To liquid ammonia (50 ml) containing MeOH (1 ml) was added mutilin (625 mg) in THF (6 ml), followed by Li (100 mg) in small portions. After stirring for 10 min, the reaction was worked up and the product chromatographed on alumina. Elution with benzene–chf (3:1) gave the known α-dihydrotriol (88 mg) identified by direct comparison.

Further elution with chf and crystallization from aqueous MeOH yielded *mutilin β-dihydrotriol* (396 mg), m.p. 196–199°, $\nu_{max}$ (in chf) 3410, 3366, and 3302 $cm^{-1}$. (Found: C, 73·8; H, 11·0. $C_{20}H_{36}O_3$ requires: C, 74·0; H, 11·2%). This triol (600 mg) with $Ac_2O$–pyridine on the steam-bath for 4 hr gave the *β-dihydrotriol triacetate* (405 mg), m.p. 175–177° from ether–light petroleum after chromatography in benzene on alumina, $\nu_{max}$ (in Nujol) 1720 $cm^{-1}$. (Found: C, 69·6; H, 9·4; M (Rast), 425. $C_{26}H_{42}O_6$ requires: C, 69·3; H, 9·4%; M, 450). Oxidation of the β-dihydrotriol with $CrO_3$ in acetone afforded dihydromutilin trione, identified by direct comparison.

*Hydroxymethylene derivative of mutilin*

Mutilin (150 mg) and MeONa (75 mg) in benzene (25 ml) containing HCOOMe (7·5 ml) were stirred at room temp for 5 hr. Dilution with ice and water, acidification and extraction with ether gave *hydroxymethylene mutilin* (59%), double m.p. 116–118° (dec) and 193–198° from AcOEt–light petroleum after chromatography on silica, $\nu_{max}$ (in chf) 3600, 3540, 3400–2800, 1726, 1676, and 1613 $cm^{-1}$, $\lambda_{max}$ 268 m$\mu$ ($\epsilon$ 8,600) shifted reversibly in alkali to $\lambda_{max}$ 308 m$\mu$ ($\epsilon$ 20,600). (Found: C, 70·1; H, 9·3. $C_{21}H_{32}O_4$, $\frac{1}{2}$ $C_2H_4O_2$ requires: C, 70·4; H, 9·3%). The maximum at 1726 $cm^{-1}$, removed on evaporation with chf, confirmed the presence of AcOEt of crystallization.

The hydroxymethylene derivative (61 mg) in 2 N methanolic KOH (4 ml) was oxidized with $H_2O_2$ (2 ml, 100 vols) at room temp. The UV max at 308 m$\mu$ disappeared in 30 min, and after 2 hr the excess of oxidant was destroyed with Pd–C. The acidic product (54 mg) isolated in the usual way was oxidized further in acetone (8 ml) with 0·8 N $CrO_3$ in 9 N $H_2SO_4$ (uptake 1·75 0). The resulting acid gum (48 mg) (Found: equiv., 195. Dicarboxylic acid requires: equiv., 182) on pyrolysis at 160° for 10 min afforded a neutral oil, $\nu_{max}$ (in chf) 1802, 1756, and 1702 $cm^{-1}$.

*Alkaline autoxidation of mutilin diacetate*

The finely-powdered diacetate (305 mg), suspended in t-butyl alcoholic N potassium t-butoxide (20 ml), was shaken under $O_2$ for 7 min (1 mole uptake). After the addition of water (100 ml) and 6 N HCl (15 ml), the product was extracted into ether, which was then washed with NaOH aq. Acidification and recovery with chf gave the *diacetoxyketocarboxylic acid* (290 mg), m.p. 187–188°

from ether–light petroleum, $\nu_{max}$ (in Nujol) 3500–2600, 1724 (with shoulders), 1689, 1637, and 920 $cm^{-1}$. (Found: C, 66·2; H, 8·3; equiv., 425. $C_{24}H_{36}O_7$ requires: C, 66·1; H, 8·3%; equiv., 436). It gave a negative iodoform reaction.

Reaction of the acid (30 mg) in benzene (5 ml) with sodium azide (6·7 mg) and $H_2SO_4$ (0·6 ml) at 67° for 10 min released $CO_2$ (0·8–0·9 mole), collected as $BaCO_3$. (Found: r.m.a. × $10^{-4}$, 0·24 and 14·1 from [1-$^{14}$C]acetate- and [2-$^{14}$C]mevalonate-derived material respectively).

*Alkaline autoxidation of mutilin hydroxy-A-dione acetate*

The acetate (105 mg) in t-butyl alcoholic N potassium t-butoxide was shaken with $O_2$ for 6 min (1 mole uptake). The product, worked up as above, was the *acetoxydiketocarboxylic acid* (89 mg), m.p. 196–199° from ether–pentane, $\nu_{max}$ (in Nujol) 3200–2500, 1730, and 1708 $cm^{-1}$. (Found: C, 67·0; H, 8·0. $C_{22}H_{32}O_6$ requires: C, 67·3; H, 8·2%).

*Oxidation of dihydromutilin trione with selenium dioxide*

The dihydrotrione (320 mg) and $SeO_2$ (400 mg) were heated in refluxing AcOH (16 ml) for 2 hr. On cooling, the soln was filtered from Se and evaporated under reduced press. An ethereal extract of the residue was washed successively with $NaHCO_3$ aq, $Na_2CO_3$ aq, and 1 N NaOH.

The hydroxide-soluble fraction was acidified and extracted with ether. The recovered material (120 mg) after chromatography on Florex and crystallization from ether–light petroleum, gave the *tetraone* (89 mg), m.p. 200–204°, $\nu_{max}$ 1712, 1708 (sh), and 1664 $cm^{-1}$, $\lambda_{max}$ 269 mμ (ε 12,700) shifted reversibly in alkali to $\lambda_{max}$ 303 (ε 7,500) and 320 mμ (infl., ε 5800). (Found: C, 72·4; H, 8·2. $C_{20}H_{28}O_4$ requires: C, 72·3; H, 8·5%). It gave an immediate violet ferric test in MeOH.

The carbonate-soluble fraction similarly yielded material (78 mg) which was chromatographed on Florex. Elution with benzene–ether (5:1) afforded the *pentaone* (53 mg), m.p. 252–253° from benzene, $\nu_{max}$ (in Nujol) 3360, 1760, 1718, 1701, and 1630 $cm^{-1}$, $\lambda_{max}$ 355 mμ (ε 4,700) shifting reversibly in alkali to $\lambda_{max}$ 443 mμ (ε 4,100). (Found: C, 69·6; H, 7·5. $C_{20}H_{26}O_5$ requires: C, 69·4; H, 7·6%). It gave an immediate red ferric test in MeOH. The same pentaone, identified by direct comparison, resulted (67%) from oxidation of the above tetraone with $SeO_2$ in boiling AcOH for 3 hr. Acetylation of the pentaone with $Ac_2O$–pyridine at room temp overnight furnished the corresponding acetate, $\nu_{max}$ (in Nujol) 1775, 1760, 1733, 1706, and 1613 $cm^{-1}$, $\lambda_{max}$ 289 mμ (ε 5,000).

The pentaone (60 mg) and Zn dust (300 mg) were heated in refluxing AcOH (5 ml) for 3 hr. The filtered mixture, after dilution with water and neutralization with $NaHCO_3$, was extracted with ether. Chromatography of the extract on Florex gave, on elution with benzene–ether (10:1), the above tetraone (72%), identified by direct comparison after crystallization from ether–light petroleum.

The pentaone (60 mg) and *o*-phenylenediamine (24 mg) in AcOH (5 ml) were heated under reflux for 20 min. Evaporation of the solvent left a residue, soluble in 1 N NaOH, which on crystallization from ether–light petroleum afforded the *quinoxaline derivative* (IX; 48 mg), m.p. 228–231°, $\nu_{max}$ 3480, 3400–2800, and 1702 $cm^{-1}$, $\lambda_{max}$ 221, 249, 265 (infl), and 352 mμ (ε 29,000, 17,000, 14,000, and 15,700 respectively), changed by addition of alkali to $\lambda_{max}$ 234, 295, and 352 mμ (ε 30,100, 11,200, and 9700 respectively). (Found: C, 75·0; H, 7·0; N, 6·7. $C_{26}H_{30}O_3N_2$ requires: C, 74·6; H, 7·2; N, 6·7%). Treatment with $Ac_2O$–pyridine (1:1) on the steam-bath for 30 min yielded the *quinoxaline acetate*, m.p. 205–208° from ether–light petroleum, $\nu_{max}$ (in $CS_2$) 1771 and 1703 $cm^{-1}$. (Found: C, 73·3; H, 6·7. $C_{28}H_{32}O_4N_2$ requires: C, 73·0; H, 7·0%).

*Oxidation of mutilin diacetate with selenium dioxide*

The diacetate (280 mg) was oxidized with $SeO_2$ (400 mg) and the product fractionated as for dihydromutilin trione. The hydroxide-soluble material was chromatographed on Florex, benzene–ether (10:1) eluting the *diketodiacetate* (72 mg), m.p. 176–178° from light petroleum, $\nu_{max}$ 3400, 3065, 1729, 1704, and 1647 $cm^{-1}$, $\lambda_{max}$ 268 mμ (ε 12,000) changed reversibly by alkali to $\lambda_{max}$ 309 mμ (ε 8,900). (Found: C, 68·7; H, 8·4. $C_{24}H_{34}O_6$ requires: C, 68·9; H, 8·2%).

The carbonate-soluble fraction after chromatography on Florex and elution with benzene–ether (5:1) gave the *triketodiacetate* (61 mg), m.p. 187–190° from ether-light petroleum, $\nu_{max}$ (in Nujol) 3600–3200, 1760, 1738 (sh), 1728, 1705 (sh), and 1638 $cm^{-1}$, $\lambda_{max}$ 343 mμ (ε 4,600) changed reversibly by alkali to $\lambda_{max}$ 443 mμ (ε 3,700). (Found: C, 66·5; H, 7·4; OAc, 17·9. $C_{24}H_{32}O_7$ requires: C, 66·7; H, 7·5; OAc, 19·9%). Reduction of this triketodiacetate with Zn, as described for the analogous pentaone above, afforded the corresponding diketodiacetate (64%), identified by direct comparison.

*Bisnor-mutilin hydroxy-A-dione*

Mutilin hydroxy-A-dione (150 mg) in AcOEt (15 ml) was ozonized to completion at 0°. $H_2O_2$ (2 ml, 100 vols) was added and the AcOEt removed under reduced press. Working up in the usual way gave acidic material (118 mg), $\nu_{max}$ 3480, 3500–2600, 1732, and 1702 cm$^{-1}$, which did not crystallize. Heated under reflux in sat $Na_2CO_3$ aq for 6 min, this material (18 mg) afforded a neutral gum (12 mg), $\nu_{max}$ 3625, 1738, and 1701 cm$^{-1}$.

The acidic material (65 mg) was heated at 130° for 6 min under N, releasing $CO_2$ (0·94 mole) collected as $BaCO_3$. The neutral residue was separated into 2 components by gradient elution from Florex with ether–benzene (0–10%). The first fraction (27 mg) failed to crystallize, but showed IR absorption very similar to that of the second fraction (25 mg), *bisnor-mutilin hydroxy-A-dione*, m.p. 127–128° from ether–light petroleum, $\nu_{max}$ 3625, 1739, and 1700 cm$^{-1}$. (Found: C, 74·2; H, 9·6. $C_{18}H_{28}O_3$ requires: C, 74·0; H, 9·7%).

*Selenium dehydrogenation of the β-dihydrotriol triacetate*

The β-dihydrotriol triacetate (800 mg) was heated with Se powder (1·6 g) at 300–310° for 1 hr. On cooling, the mixture was extracted with ether and the extract washed with $NaHCO_3$ aq to remove AcOH. The brown gum left on evaporation of the ether was heated with Se (1 g) at 290–300° for 16 hr. On cooling, the powdered solid was extracted with ether. Chromatography of the extract (230 mg) on alumina (Spence Grade "O") in light petroleum gave first material (97 mg) showing weak benzenoid UV absorption, followed by a fluorenoid fraction (123 mg). The latter material was re-chromatographed on alumina in light petroleum, collecting 10 ml fractions. Fractions 15–17 afforded a crystalline $C_{18}$-fluorene (41 mg), purified by vacuum sublimation and recrystallization from MeOH, m.p. 151·5–155°, $\lambda_{max}$ (in hexane) 261, 271, and 301 mμ (ε 18,600, 16,600, and 4000 respectively). (Found: Mol. wt. (MS), 236. $C_{18}H_{20}$ requires: Mol. wt. 236). Non-crystalline fractions (18 mg) were combined, oxidized with $KMnO_4$ (54 mg) in acetone (3 ml) at room temp for 3 hr, and the yellow product (18 mg) chromatographed on alumina. Elution with ether (3%) in light petroleum yielded one crystalline fraction, m.p. 122·5–126° after vacuum sublimation and recrystallization from light petroleum, $\lambda_{max}$ 256 and 264 mμ (ε 57,000 and 72,000), shown by mass spectroscopy to be a mixture of fluorenones. (Found: Mol. wts. 278, 264, and 250. $C_{20}H_{22}O$, $C_{19}H_{20}O$, and $C_{18}H_{18}O$ require respectively: Mol. wts. 278, 264, and 250).

*Deoxymutilin*

Mutilin (159 mg) and KOH (180 mg) were heated under reflux in diethylene glycol (4 ml) and hydrazine hydrate (0·4 ml, 99%) for 2 hr. The mixture was distilled until the solution's temp reached 193°, and refluxing was then continued for 2 hr. Working up in the usual manner gave a neutral product (102 mg), which was chromatographed on alumina. Elution with benzene–ether (10:1) gave *deoxymutilin*, m.p. 137–139° from light petroleum, $\nu_{max}$ (in $CS_2$) 3648, 3080, and 917 cm$^{-1}$. (Found: C, 78·5; H, 11·2. $C_{20}H_{34}O_2$ requires: C, 78·4; H, 11·2%).

Oxidation of deoxymutilin (10 mg) in acetone (4 ml) with 0·8N $CrO_3$ (2 mols) in 9N $H_2SO_4$ gave deoxymutilin dione, m.p. 67–68°, $\nu_{max}$ 3040, 1697, 1630, and 921 cm$^{-1}$. (Found: Mol. wt. (MS), 302. $C_{20}H_{30}O_2$ requires: mol. wt. 302).

*Reaction of mutilin hydroxy-A-dione with strong base*

The dione (150 mg) was heated under reflux in 7N methanolic KOH (15 ml) for 20 min under N. Dilution with water and extraction with ether gave a product (141 mg) which was chromatographed on alumina. Elution with benzene–ether (5:2) yielded the cyclic hemiketal (partial structure XXIII; 104 mg) a colourless gum, $\nu_{max}$ 3480, 3075, 1696, 1626, and 910 cm$^{-1}$, $\lambda_{max}$ 236 mμ (ε 13,500), which failed to crystallize after repeated chromatography or vacuum sublimation, and for which reliable analyses could not be obtained. It gave a negative iodoform reaction, and was recovered (identified by IR and UV spectra) after treatment with $CrO_3$ in pyridine at 0° overnight.

The hemiketal XXIII (50 mg) with 2,4-dinitrophenylhydrazine hydrochloride (45 mg) in anhydrous MeOH (8 ml) at room temp for 14 hr gave red crystals (59 mg), which were purified by filtration in chf through bentonite-kieselguhr (4:1). Recrystallization from ether–MeOH, and from light petroleum, gave the *O-methyl 2,4-dinitrophenylhydrazone* (24 mg), double m.p. 138° and 156–162°, $\nu_{max}$ 3220, 3015, 1618, and 1592 cm$^{-1}$, $\lambda_{max}$ 391 mμ (ε 31,500). (Found: C, 63·4; H, 7·1; N, 10·8; OMe, 6·5. $C_{26}H_{33}O_5N_4,OCH_3$ requires: C, 63·3; H, 7·1; N, 11·0; OMe, 6·1%).

The hemiketal XXIII (42 mg) was warmed in $Ac_2O$–pyridine (1:2, 6 ml) on the steam-bath for 3 hr. Chromatography of the product on alumina and elution with ether (3%) in benzene afforded the dehydration product (partial structure XVIII) as a gum (30 mg), $\nu_{max}$ 3080, 1680, 1625, and 911 $cm^{-1}$, $\lambda_{max}$ 236 m$\mu$ ($\epsilon$ 13,600). Ozonolysis of this dehydration product (10 mg) in AcOH yielded equal amounts of formaldehyde and acetaldehyde, separated by steam distillation and examined by paper chromatography[52] of their 2,4-dinitrophenylhydrazones ($R_F$ 0·18 and 0·23 respectively).

The hemiketal XXIII (35 mg) in THF (1·5 ml) was added to stirred liquid ammonia (10 ml), followed by Li (10 mg). After 20 min the excess of Li was destroyed with MeOH (0·6 ml), and the reduction product (31 mg), showing only weak end-absorption in the UV, was isolated in the usual way. Without purification this was titrated in acetone with 0·8N $CrO_3$ in 9N $H_2SO_4$, to give a neutral gum (26 mg), $\nu_{max}$ 1776 and 1740 $cm^{-1}$.

*Oxidation of the hemiketal* XXIII *to the γ-lactone* XXIV

The hemiketal XXIII (75 mg) in acetone (25 ml) was titrated with 0·8N $CrO_3$ in 9N $HSO_4$. Chromatography of the neutral product on alumina and elution with benzene–ether (5:1) gave the *γ-lactone* XXIV (44 mg), m.p. 128–129°, $\nu_{max}$ 3060, 1773, 1696, 1641, 1630, and 920 $cm^{-1}$, $\lambda_{max}$ 236 m$\mu$ ($\epsilon$ 14,000). (Found: C, 74·8; H, 8·5; mol. wt. (Rast), 281. $C_{18}H_{24}O_3$ requires: C, 75·0; H, 8·4%; mol. wt. 288.) Similar oxidation of the above dehydration product XVIII afforded the same γ-lactone (79%), identified by direct comparison.

Hydrogenation (uptake 1 mole) of the γ-lactone (20 mg) in MeOH over Pt (20 mg) furnished the *dihydro-γ-lactone*, m.p. 155–156°, $\nu_{max}$ 1768, 1695, and 1627 $cm^{-1}$, $\lambda_{max}$ 236 m$\mu$ ($\epsilon$ 14,000). (Found: C, 74·7; H, 9·0. $C_{18}H_{26}O_3$ requires: C, 74·5; H, 9·0%).

*Monobenzylidene derivatives of mutilin hydroxy-diones*

Mutilin hydroxy-A-dione (48 mg) and benzaldehyde (1 ml) in 7N methanolic KOH (4 ml) were stood at room temp for 15 min. Chromatography of the product on alumina and elution with benzene–ether (10:1) yielded *monobenzylidene mutilin hydroxy-A-dione* (38 mg), m.p. 202–203° $\nu_{max}$ (in Nujol) 3540, 1710, 1696, 1630, and 921 $cm^{-1}$, $\lambda_{max}$ 295 m$\mu$ ($\epsilon$ 21,000). (Found: C, 79·6; H, 8·3. $C_{27}H_{34}O_3$ requires: C, 79·8; H, 8·5%). Treatment of this derivative with 7N methanolic KOH on the steam-bath for 20 min gave a neutral gum, $\lambda_{max}$ 307 m$\mu$ ($\epsilon$ 7,000).

The hydroxy-B-dione similarly afforded *monobenzylidene mutilin hydroxy-B-dione* (68%), m.p. 245–247°, $\nu_{max}$ (in Nujol) 3610, 1717, 1685, 1627, and 908 $cm^{-1}$, $\lambda_{max}$ 295 m$\mu$ ($\epsilon$ 20,500). (Found: C, 79·5; H, 8·3. $C_{27}H_{34}O_3$ requires: C, 79·8; H, 8·5%). This derivative was recovered after treat ment with hot 7N methanolic KOH for 20 min.

*Reaction of mutilin trione with strong base*

The product (191 mg) from treatment of the trione (210 mg) with methanolic KOH, as described for mutilin hydroxy-A-dione, was chromatographed on alumina. Elution with benzene–ether (7:1) gave the *diene-trione* (partial structure XXVIII; 96 mg), a gum purified by re-chromatography and molecular distillation at 120°/0·5 mm, $\nu_{max}$ 1699 (broad), 1638, and 916 $cm^{-1}$, $\lambda_{max}$ 236 m$\mu$ ($\epsilon$ 13,200). (Found: C, 76·4; H, 8·8. $C_{20}H_{28}O_3$ requires: C, 75·9; H, 8·9%). This product (25 mg) was recovered (identified by IR and UV spectra) after treatment with Zn (100 mg) in refluxing AcOH (1·5 ml) for 30 min.

Further elution of the alumina chromatogram with benzene–ether (5:1) yielded the *triene-dione* (partial structure XXX; 65 mg), m.p. 126–127° from ether–light petroleum, $\nu_{max}$ 1696, 1637, 1616, and 910 $cm^{-1}$, $\lambda_{max}$ 243 m$\mu$ ($\epsilon$ 24,000). (Found: C, 80·7; H, 8·9; mol. wt. (Rast), 290. $C_{20}H_{26}O_2$ requires: C, 80·5; H, 8·8%; mol wt 298). The same triene-dione (identified by direct comparison) also resulted (72%) on further treatment of XXVIII with 7 N methanolic KOH on the steam-bath for 25 min. Hydrogenation (uptake 1 mole) of the triene-dione (30 mg) in MeOH (8 ml) over Adam's catalyst (30 mg) gave the corresponding *dihydro derivative*, m.p. 113–115°, $\nu_{max}$ 1697, 1641, and 1623 $cm^{-1}$, $\lambda_{max}$ 243 m$\mu$ ($\epsilon$ 23,500). (Found: C, 78·1; H, 9·4. $C_{20}H_{28}O_2$ requires: C, 80·0; H, 9·4%).

The triene-dione XXX (33 mg) was reduced with Li in liquid ammonia as described for XXIII. Without purification, the product (29 mg) was titrated in acetone with 0·8N $CrO_3$ in 9 N $H_2SO_4$ (uptake 1·6 0), affording a neutral gum, $\nu_{max}$ 1738 $cm^{-1}$, lacking intense UV absorption.

*Dibenzylidene derivatives of the triene-dione* XXX *and the hemiketal* XXIII

The triene-dione XXX (40 mg) and benzaldehyde (2 ml) in 7 N methanolic KOH (6 ml) were heated on the steam-bath for 15 min. Chromatography of the product on alumina gave the *dibenzylidene derivative* (38 mg), m.p. 218–220° from ether, $\nu_{max}$ (in Nujol) 1687, 1682, 1637, 1627, and 912 $cm^{-1}$, $\lambda_{max}$ 244, 268, and 355 m$\mu$ ($\epsilon$ 27,000, 22,000, and 27,300 respectively). (Found: C, 86·0; H, 7·2. $C_{34}H_{34}O_2$ requires: C, 86·0; H, 7·3%).

The hemiketal XXIII similarly yielded an amorphous derivative, $\nu_{max}$ (in $CS_2$) 3590, 1682, 1637, and 915 $cm^{-1}$, $\lambda_{max}$ 244, 271, and 355 m$\mu$ ($\epsilon$ 15,500, 17,500 and 25,500 respectively).

*Controlled chromic acid oxidation of alkaline degradation products*

Controlled oxidation of XXVIII with chromic acid[28] afforded acetic and propionic acids, identified by paper chromatography[49] of their ammonium salts ($R_F$ 0·13 and 0·22 respectively). Similar treatment of XXIII gave acetic and propionic acids, but XXX and XXIV yielded AcOH only.

*Alkaline autoxidation of mutilin trione*

Mutilin trione (200 mg) in t-butyl alcoholic potassium t-butoxide (12 ml, 1 N) was shaken with $O_2$ for 5 min (uptake 1 mole). Water (12 ml) was added and the soln heated on the steam-bath for 20 min under N. Acidification gave the *diketodicarboxylic acid* (XXVII; 147 mg), m.p. 280–282° from MeOH–chf, $\nu_{max}$ (in Nujol) 3400–2500, 1705, 1636, and 910 $cm^{-1}$, $\lambda_{max}$ 300 m$\mu$ ($\epsilon$ 145). (Found: C, 65·5; H, 8·3; equiv., 175. $C_{20}H_{30}O_6$ requires: C, 65·6; H, 8·3%; di-acid equiv., 183). The acid gave a negative iodoform reaction, and was recovered (78%) after heating at 282–285° for 3 min.

The *dimethyl ester*, prepared with ethereal diazomethane, had m.p. 188–190° from ether, $\nu_{max}$ 1730, 1715, 1625, and 915 $cm^{-1}$. (Found: C, 66·8; H, 8·7. $C_{22}H_{34}O_6$ requires: C, 67·0; H, 8·7%).

*Contraction of the medium ring*

(i) *Pleuromutilin.* Powdered pleuromutilin (150 mg) and $PCl_5$ (300 mg) suspended in dry benzene (7·5 ml) and light petroleum (7·5 ml) were stirred at room temp for 1 hr. Ice and water were added, and the organic layer separated, dried, and evaporated. Chromatography of the residue on alumina afforded, on elution with benzene, the *dienechloroacetate* (XLI; 74 mg), m.p. 189–192° from benzene–light petroleum, $\nu_{max}$ (in Nujol) 3065, 1747, 1732, 1635, 922, and 895 $cm^{-1}$, $\lambda_{max}$ 226 m$\mu$ ($\epsilon$ 16,200). (Found: C, 69·7; H, 8·1. $C_{22}H_{31}ClO_3$ requires: C, 69·8; H, 8·2%).

The dienechloroacetate (44 mg) in AcOEt (10 ml) at 0° was ozonized to completion. After the addition of $H_2O_2$ (1 ml, 100 vols) and removal of the AcOEt under reduced press, working up in the usual way gave the *trisnorcarboxylic acid* (XLII; 14 mg), m.p. 190–193° after several crystallizations from ether–light petroleum, $\nu_{max}$ 3500–2500, 1740, and 1709 $cm^{-1}$. (Found: C, 61·3; H, 7·4. $C_{19}H_{27}ClO_5$ requires C, 61·6; H, 7·3%). Ozonolysis of the dienechloroacetate (23 mg) in AcOH (5 ml), followed by the addition of Zn (50 mg) and water (15 ml) and steam-distillation, yielded formaldehyde, collected as the 2,4-dinitrophenylhydrazone (18 mg, 1·4 moles) and identified by direct comparison.

(ii) *Dihydropleuromutilin.* Similar treatment of dihydropleuromutilin (150 mg) with $PCl_5$ (300 mg), chromatography of the product on alumina and elution with benzene, gave the *monoenechloroacetate* (XLIII; 69 mg), m.p. 145–147° from ether–light petroleum, $\nu_{max}$ (in $CS_2$) 3050, 1745, 1733, 1633, and 892 $cm^{-1}$. (Found: C, 69·1; H, 8·8. $C_{22}H_{33}ClO_3$ requires: C, 69·4; H, 8·7%).

Ozonolysis of the monoenechloroacetate (45 mg) in AcOH (5 ml), followed by steam-distillation, gave formaldehyde, isolated as the 2,4-dinitrophenylhydrazone (0·6 mole), identified by direct comparison. (Found: r.m.a., 0, from [1-$^{14}$C]acetate- or [2-$^{14}$C]mevalonate-derived material). Neutralization of the residual liquors after steam-distillation and extraction with ether afforded the *norketone* (XLIV; 33 mg), m.p. 175–178° from ether, $\nu_{max}$ (in Nujol) 1738, 1730, and 1702 $cm^{-1}$. (Found: C, 65·1; H, 8·1. $C_{21}H_{31}ClO_4$ requires: C, 65·6; H, 8·2%). The norketone gave a negative ferric test.

*Intramolecular aldol cyclizations*

Bisnor-mutilin hydroxy-A-dione (50 mg) was heated in 1 N methanolic KOH on the steam-bath for 15 min (optimum time). Crystallization of the product from chf gave the *dihydroxyketone* XLVI (R=H, OH; 28 mg), m.p. 222–223°, $\nu_{max}$ (in Nujol) 3385, 3307, and 1706 $cm^{-1}$. (Found: C, 73·7; H, 9·5. $C_{18}H_{28}O_3$ requires: C, 74·0; H, 9·7%).

This dihydroxyketone (23 mg) in acetone (6 ml) was titrated with 0·8N $CrO_3$ in 9N $H_2SO_4$ (uptake 1 0), yielding the *hydroxydione* XLVI (R=O; 14 mg), m.p. 166–168° from ether–light petroleum, $\nu_{max}$ 3590 and 1706 cm$^{-1}$, $\lambda_{max}$ 294 mμ (ε 75). (Found: C, 74·4; H, 9·1; mol wt (Rast), 290. $C_{18}H_{26}O_3$ requires: C, 74·5; H, 9·1%; mol wt 290).

Nor-mutilin trione carboxylic acid (50 mg) was heated under reflux in 1N methanolic KOH for 15 min. Chromatography of the product on alumina and elution with benzene–ether (5:1) afforded XLVI (R=O; 28 mg), identified by direct comparison.

*Transannular hydride migrations*

(i) *The acetoxyketo-δ-lactone* XLVIII (R=CH=$CH_2$). The diacetoxyketocarboxylic acid XLVII (R=Ac; 100 mg) in t-butyl alcoholic N potassium t-butoxide (15 ml) was stood at room temp for 1 hr under N. The white ppt obtained on dilution with water and acidification was extracted with ether. The ether extract was washed with NaOH aq and then water, and evaporated to yield the *acetoxyketo-δ-lactone* XLVIII (R=CH=$CH_2$; 39 mg), m.p. 205–206° from EtOH, $\nu_{max}$ 3075, 1757, 1740, 1700, 1631, and 927 cm$^{-1}$, $\lambda_{max}$ 301 mμ (ε 90). (Found: C, 70·1; H, 8·6. $C_{22}H_{32}O_5$ requires: C, 70·2; H, 8·6%).

The acetoxyketo-δ-lactone (35 mg) in AcOEt (8 ml) was ozonized to completion. The product from oxidative work-up with $H_2O_2$ (1 ml, 100 vols) was separated into acidic (24 mg) and neutral (9 mg) fractions, neither of which crystallized. The acidic fraction, $\nu_{max}$ 3500–2600, 1755, 1742, and 1706 cm$^{-1}$, was heated at 120° for 7 min under N. Evolved $CO_2$ (0·95 mole) was collected as $BaCO_3$, whilst crystallization of the neutral residue from ether–pentane gave the *bisnor-δ-lactone* XLVIII (R=H; 10 mg), m.p. 159–163°, $\nu_{max}$ 1756, 1740, and 1704 cm$^{-1}$. (Found: C, 68·4; H, 8·7. $C_{20}H_{30}O_5$ requires: C, 68·6; H, 8·7%).

(ii) *The anhydro compound* L (R=O). The dihydromutilin monoacetate XLIX (R=O; 300 mg) in pyridine (15 ml) containing $POCl_3$ (3 ml) was left at room temp overnight. The mixture was poured on to ice, extracted with ether, and the extract washed successively with dil HCl, $Na_2CO_3$ aq, and water. Chromatography of the neutral product on alumina gave, on elution with benzene, the *anhydro compound* L (R=O; 192 mg), m.p. 146–148° from light petroleum, $\nu_{max}$ (in $CS_2$) 3060, 1732, and 1627 cm$^{-1}$, end absorption λ 214 mμ (ε 240). (Found: C, 76·2; H, 9·7. $C_{22}H_{34}O_3$ requires: C, 76·3; H, 9·9%). Ozonolysis of this anhydro compound (43 mg) in AcOH for 20 min, followed by the addition of water and steam-distillation into 2,4-dinitrophenylhydrazine in dil HCl yielded no 2,4-dinitrophenylhydrazone.

*Retro-aldol fission to the keto-acid hemi-acetal* LI (R=H, OH)

The diacetoxyketocarboxylic acid XLVII (R=Ac; 200 mg) in 1N NaOH (10 ml) was heated under reflux for 30 min. Acidification, extraction with chf, and evaporation left an acidic gum which crystallized on trituration with ether–pentane. Recrystallization from ether–pentane afforded the *keto-acid hemi-acetal* LI (R=H, OH; 84 mg), m.p. 128–132°, $\nu_{max}$ 3370, 3500–2600, 1704, 1638, and 921 cm$^{-1}$, $\lambda_{max}$ 295 mμ (ε 74). (Found: C, 68·5; H, 9·2. $C_{20}H_{32}O_5$ requires: C, 68·2; H, 9·2%). Treatment with ethereal diazomethane gave the corresponding Me ester, a gum, $\nu_{max}$ 3580, 1739, 1698, and 922 cm$^{-1}$.

The Me ester (150 mg) in acetone (25 ml) was treated with 8N $CrO_3$ in 9N $H_2SO_4$ (0·75 ml) at 10° for 15 min. The excess of oxidant was destroyed with isopropanol and the reaction worked up in the usual way, affording acidic (69 mg) and neutral (78 mg) fractions, neither of which crystallized. The neutral fraction, $\nu_{max}$ 1782, 1737, 1697, and 927 cm$^{-1}$, was hydrolysed with 0·5N methanolic KOH (5 ml) under reflux for 15 min. Dilution with water and acidification gave the *keto-acid γ-lactone* LI (R=O; 34 mg), m.p. 143–144° from ether–pentane, $\nu_{max}$ 3500–2600, 1782, 1702, 1639, and 929 cm$^{-1}$. (Found: C, 68·7; H, 8·7. $C_{20}H_{30}O_5$ requires: C, 68·6; H, 8·7%).

The acidic fraction (65 mg), $\nu_{max}$ 3500–2500, 1740, 1700, 1638, and 928 cm$^{-1}$, from the oxidation was heated under reflux in 0·25N aqueous KOH (5 ml) for 6 min. Acidification furnished the *dicarboxylic acid* LII (39 mg), m.p. 213–215° from chf–light petroleum, $\nu_{max}$ (in Nujol) 3500–2600, 1740, 1702, and 1634 cm$^{-1}$, showing no high-intensity UV absorption. (Found: C, 62·9; H, 8·1; equiv., 175. $C_{20}H_{30}O_7$ requires: C, 62·8; H, 7·9%; di-acid equiv., 191). The same dicarboxylic acid, identified by direct comparison, was the only isolable product (40%) from oxidation of LI (R=H, OH) with $CrO_3$ in acetone.

The dicarboxylic acid LII (42 mg) in 1N NaOH (5 ml) was heated under reflux for 20 min. Acidification with phosphoric acid and steam-distillation gave formic and tiglic acids, identified by paper chromatography[49] as their ammonium salts ($R_F$ 0·12 and 0·43 respectively). Ether extraction of the distillate afforded tiglic acid (8·5 mg), m.p. 62–63° from water, identified by direct comparison and conversion into the *p*-bromophenacyl ester, m.p. and mixed m.p. 68–69°. The residual liquors after steam-distillation were extracted with ether to yield the *hydroxyketocarboxylic acid* LIII (R = H, OH), m.p. 176–178° from ether, $\nu_{max}$ (in Nujol) 3500–2500, 3250, and 1730 $cm^{-1}$, showing no intense UV absorption. (Found: C, 66·4; H, 8·9; equiv., 245. $C_{14}H_{22}O_4$ requires: C, 66·1; H, 8·7%; equiv., 254). Oxidation with $CrO_3$ in acetone gave the corresponding *diketocarboxylic acid* LIII (R = O), m.p. 123–125°, $\nu_{max}$ 3500–2500, 1727, and 1713 $cm^{-1}$. (Found: C, 66·5; H, 7·9. $C_{14}H_{20}O_4$ requires: C, 66·7; H, 8·0%).

This diketocarboxylic acid LIII (R = O; 18 mg) was heated in refluxing 1N NaOH for 10 min. The product was sublimed at 185°/0·5 mm and separated into an acidic fraction (12 mg) (Found: equiv., 146; $C_{14}H_{22}O_5$ requires: di-acid equiv., 135) and a neutral fraction (4 mg), $\nu_{max}$ 1806, 1762, and 1705 $cm^{-1}$.

*Alkaline autoxidation of mutilin hydroxy-*B*-dione acetate*

The acetoxy-B-dione (120 mg) in N t-butyl alcoholic potassium t-butoxide (8 ml) was shaken with $O_2$ for 5 min (uptake 1 mole). Water (8 ml) was added and the mixture warmed on the steam-bath for 20 min. Acidification, extraction with ether and crystallization from ether–pentane gave LI (R = O; 89 mg), identified by direct comparison.

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