

## THE STRUCTURE OF TERREMUTIN<sup>1</sup>

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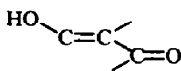
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(Received in USA 2 December 1967; accepted for publication 14 February 1968)

**Abstract**—Terremutin, a new metabolite isolated from cultures of an *Aspergillus terreus* mutant, has been characterized as 4,5-dihydroxy-3-methyl-7-oxabicyclo[4.1.0]hept-3-en-2-one. The complete stereochemistry is presented.

A STRAIN of the mold *Aspergillus terreus* producing high yields of itaconic acid was mutated by UV irradiation and a clone selected which produced in still fermentation not only itaconic acid but also terrein and a third substance that could not be identified chromatographically with probable known fungal metabolites.\* The new substance, now the principal product, was designated terremutin, and was isolated and characterized as 4,5-dihydroxy-3-methyl-7-oxabicyclo[4.1.0]hept-3-en-2-one 1.

The white, crystalline substance was optically active, water-soluble, acidic, and had reducing properties. A neutral equivalent of 156 confirmed a mol. wt. by mass spectrograph, and the spectrogram showed a strong secondary peak at  $m/e$  154. Elemental analysis then indicated  $C_7H_8O_4$  as the molecular formula. A Kuhn-Roth oxidation showed the presence of one C—Me group, and a MeO determination was negative. No derivatives were obtained with carbonyl reagents, coupling reagents, anhydrides, isocyanates nor diazomethane. Aqueous solutions slowly developed a blue color with ferric chloride. The IR spectrum (KBr) showed strong absorption at 3.04, 6.08 and 6.14  $\mu$ . The band at 3.04  $\mu$  was consistent with the presence of at least one OH group. The band at 6.08  $\mu$  is in the range typical of the group:



Quinonoid CO groups also absorb in this region. The band at 6.14  $\mu$  might be assigned to the conjugated C—C double bond.<sup>2</sup>

When colorless terremutin crystals were dissolved in pyridine, an amber color developed, then yellow crystals, m.p. 182–183.5° separated. The elemental analysis was consistent with a 1:1 molecular complex. Such a complex seemed indicated, too, by the UV absorption spectrum which, relative to that of uncomplexed terremutin, showed a 20  $m\mu$  bathochromic shift accompanied by a marked augmentation of the extinction coefficient.

After terremutin was dissolved in pyridine-acetic anhydride mixture at ambient temperatures, again an amber color developed but gradually faded, and after 15 hr

\* The first terremutin sample was isolated by W. J. McGahren from fermentations carried out by R. A. Fitts and J. P. Lorenzo at our New York Plant.

a white crystalline acetate, m.p. 197–198°\* precipitated. A Rast mol. wt. (336) combined with an elemental analysis showed the molecular formula to be  $C_{15}H_{16}O_8$  (mol wt 324). Acetyl determinations and saponification equivalents were impossible due to decomposition and color formation, but a Kuhn-Roth oxidation indicated 5 C—Me groups, making a tetraacetate apparent. There was no remaining active hydrogen by the Zerewitinov method.

It was obvious that acetylation of terremutin had been accompanied by extensive rearrangement to an aromatic system upon examination of the UV absorption spectrum of the acetate. In methanol there was a low, broad peak,  $\lambda_{\max}$  268  $\mu$ ,  $\epsilon$  745. A tetrahydroxytoluene had been formed followed by complete acetylation. The m.p. and UV absorption characteristics were the same as reported for 2,3,5,6-tetraacetoxytoluene 2†, Fig. 1.

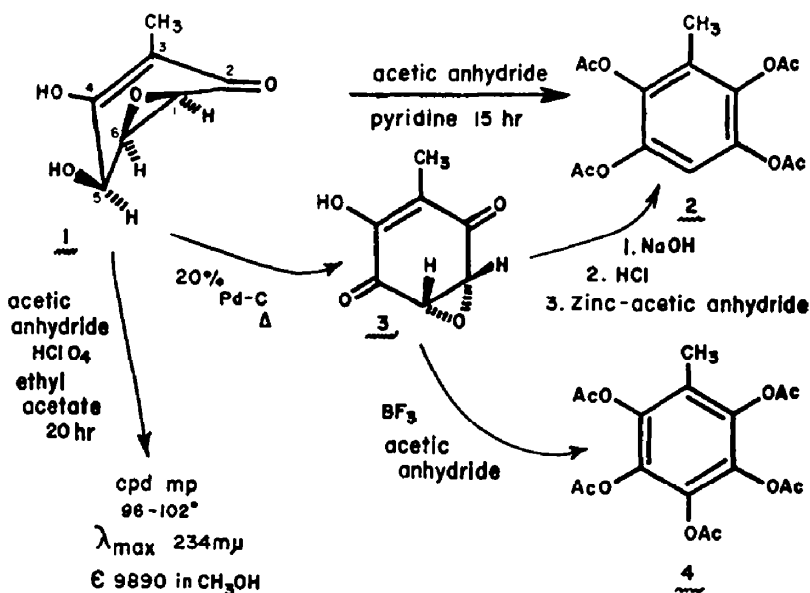


FIG. 1 Relation of terremutin to terreic acid.

At this point the published reports of the previously characterized metabolites of *Aspergillus terreus* were reexamined in the light of the carbocyclic skeleton and of the other evidence at hand concerning the structure of terremutin. Resemblances were noted to terreic acid 3, which had been converted to the same tetraacetoxytoluene by reductive acetylation.<sup>5</sup>

The NMR spectrum (in  $\tau$ ) of terremutin in deuterium oxide solution showed a singlet at 7.8 (3H) due to the Me group, a quartet with fine structure (2H) with the doublets centered at 5.6 and 5.9, corresponding to the two protons adjacent to the epoxide group, and a signal (1H) at 4.8 which moved to 5.2 and developed more fine

\* A m.p. of 198° for this substance has been reported.<sup>3</sup>

†  $\lambda_{\max}$  268,  $\epsilon$  678 for this acetate has been reported.<sup>4</sup>

structure upon addition of a few drops of triethylamine (Fig. 7). Combined with other evidence this spectrum was consistent with a dihydroterreic acid.

Although several oxidative methods were applied to terremutin unsuccessfully, it was dehydrogenated over Pd-C in a sublimation apparatus, and the resulting terreic acid collected on the cold-finger. Comparison with an authentic sample\* confirmed the identity (cf. Fig. 1).

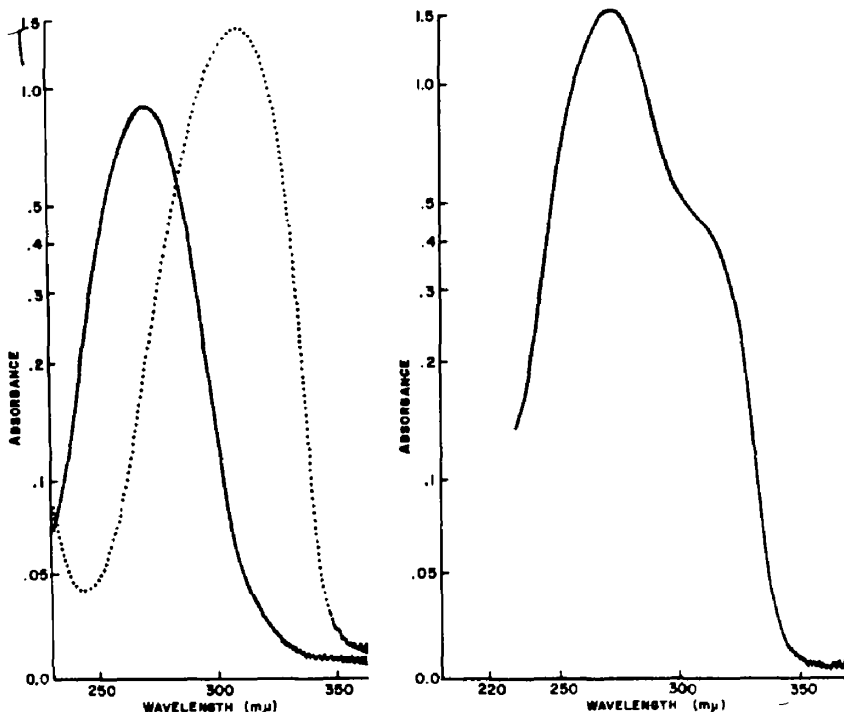


FIG. 2A UV absorption of terremutin in acidic (—) and basic (·····) methanol, conc  $3.56 \times 10^{-5}$  mole/l.

FIG. 2B UV absorption of terremutin in methanol, conc  $7.09 \times 10^{-5}$  mole/l.

Disregarding the stereochemistry, the gross structure still was not defined because of the possibility of two dihydroterreic acids, depending on whether the reduced position were at C-2 or at C-5 (cf. Fig. 4 for the alternate structures). The UV absorption spectrum (Fig. 2) proved to be more confusing than helpful in making this distinction. In neutral methanol the maximum absorption was at 272 mμ with a prominent shoulder at 310 mμ. In methanol 0.01N in hydrochloric acid the shoulder disappeared, leaving a single peak,  $\lambda_{\max}$  272 mμ,  $\epsilon$  12,900. In methanol 0.01N in sodium hydroxide the  $\lambda_{\max}$  was at 310 mμ,  $\epsilon$  20,200. The simplest interpretation of this resolution of the complex curve was to assume partial ionization in neutral methanol, suppressed by addition of acid or completed by addition of base. In structural terms this relationship

\* A sample of terreic acid was generously supplied by Professor Sheehan.

is shown in Fig. 3. We recalled, however, that in the recently reported benzene oxide-oxepin tautomerism<sup>5</sup> a compound UV absorption curve was obtained in isoöctane which was nearly superimposable on our terremutin curve in neutral methanol. There seemed to be at least a remote possibility that a similar equilibrium might arise in our case as indicated in Fig. 3. It was possible to rule out such a tautomerism in the case of terremutin by reference to its other optical properties. All optical activity would be lost in tautomerization to an oxepin-like structure as in Fig. 3, yet in basic methanol solution the optical activity of terremutin was not lost, but indeed somewhat enhanced.

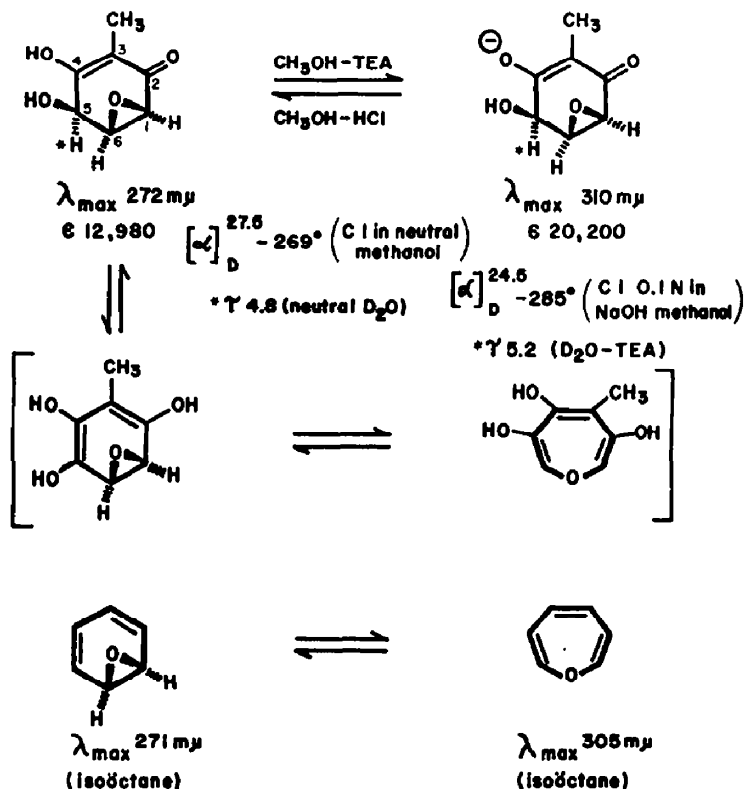


FIG. 3 Correlation of UV absorption data with specific rotations and NMR observations.

The UV absorption of terremutin is compared with that of certain pertinent structures in Fig. 4. If the alternate structure, reduced at C-2, is considered, it is seen to be a cyclohexane  $\alpha$ -diketone while terremutin is a  $\beta$ -diketone. Many well-defined structures of each type are known, and the UV absorption characteristics of a number of these have been published.<sup>†</sup> The positions of the absorption maxima of, e.g. cholestane-2,3-dione or of diosphenol correspond better with those of terremutin than do the maxima of the structurally more related  $\beta$ -diketones such as dimedone although the

<sup>†</sup> The characteristic diosphenol absorption has been discussed.<sup>6</sup>

extinction coefficients are relatively low. If the somewhat closer analogy, 2-methyl-dimedone, is considered, a  $\lambda_{\max}$  of 295  $\mu\mu$  ( $\epsilon$  24,500) has been reported<sup>7</sup> for alkaline ethanol solution, a 15  $\mu\mu$  red shift from the unsubstituted dimedone which brings it into closer alignment with terremutin.

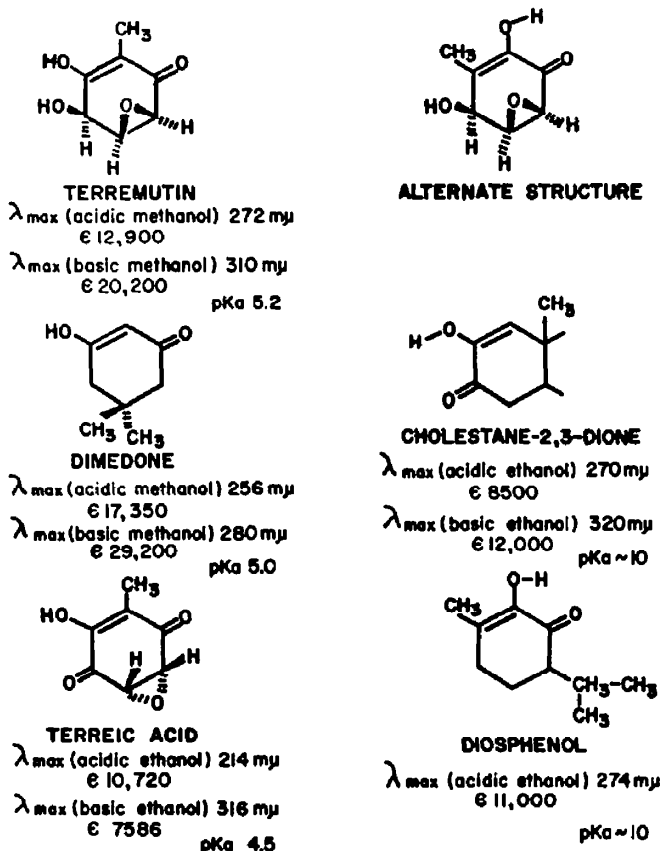
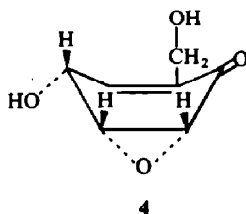


FIG. 4 Comparison of UV absorptions with acidity characteristics.

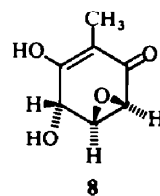
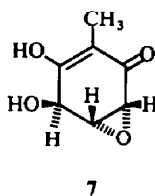
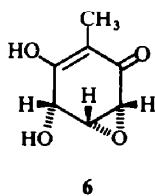
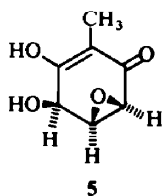
Despite the resemblance of the ultraviolet absorption maxima of terremutin to those of the  $\alpha$ -diketones, we believe that comparisons of relative acidities offer an unassailable argument for the  $\beta$ -diketone structure. It is well known that cyclohexane-1,3-diones exist largely in the half-enolized form. A recent study of dilution effects points up the contribution of intermolecular association to the stabilization of this form.<sup>8</sup> The cyclohexane-1,2-diketones also are extensively enolized, fresh 1,2-cyclohexanedione itself having been shown to be 92% in the half enolic form.<sup>9</sup> In both cases there is a marked solvent effect in solutions. The cyclohexane-1,3-diones, however, which may be envisaged as vinylogous carboxylic acids, are much more acidic than the cyclohexane-1,2-diones with  $pK_a$  values near 5 while those of the

vicinal diketones are near 10.\* This fundamental property is less subject to substituent influence than is the ultraviolet absorption spectrum. Since the  $pK_a$  of terremutin is in good agreement with those of the cyclohexane-1,3-diketones, we believe that this constitutes valid evidence for the structural assignment.

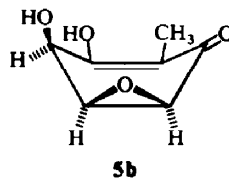
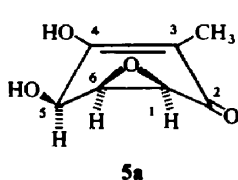
Turning to the stereochemical problem, it was necessary to select one of eight isomers. The problem was in some respects analogous to that encountered elsewhere recently in the determination of the constitution of another mold metabolite, epoxydon.<sup>11</sup> Epoxydon **4** is indeed a structural isomer of terremutin, and it undergoes aromatization when acetylated (to form a triacetate) as does terremutin. Thus, the



four diastereoisomers below must be considered :



Moreover, inspection of Dreiding models shows that two conformers (shallow incipient and perhaps slightly distorted boat forms) are possible in each case. For example, considering only the diastereoisomer **5** the conformers would be:



Of these, conformer **5a** with hydrogens at C-5 and C-6 synclinal and the hydroxyl group at C-5 quasiequatorial would be favored over conformer **5b** with the hydrogens synperiplanar and the hydroxyl group quasiaxial, particularly because of the more favorable resonance possibilities between the delocalized electrons of the oxirane

\* A list of references concerning the relative acidities of cyclohexane-1,2- and cyclohexane-1,3-diones has been discussed.<sup>10</sup>

ring with the  $\pi$ -electrons of the carbonyl group.\* Indeed this interaction probably tends to stabilize the conformation so that the CO group is restricted slightly out of plane with the conjugated double bond. This warping, together with the resonance interaction itself, may account for the deviation of the UV absorption maxima from those of a  $\beta$ -diketone such as dimedone.† Dimedone not only does not contain the epoxide group, but also the low activation energy for conversion of one conformer to the other (about 4 Kcal/mole) permits such rapid interconversion that the conformers cannot be distinguished by the nuclear magnetic resonance spectrum at ambient temperatures.<sup>10</sup> A similar conformational assessment could be made for each of the other three diastereoisomers.

The CD spectrum of terremutin is shown in Fig. 5.‡ The  $n \rightarrow \pi^*$  CO absorption

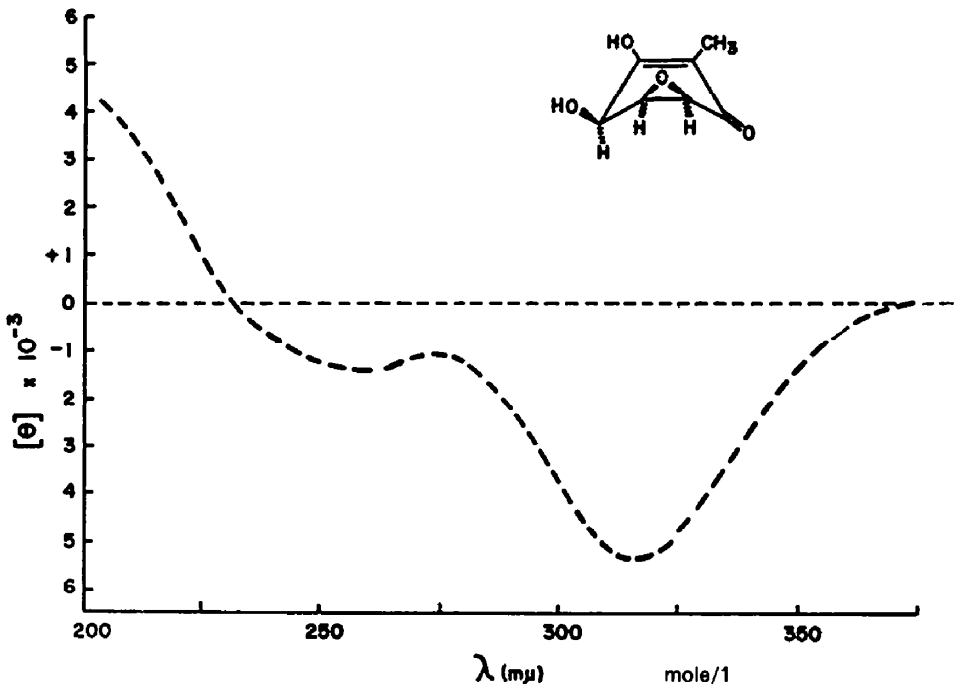


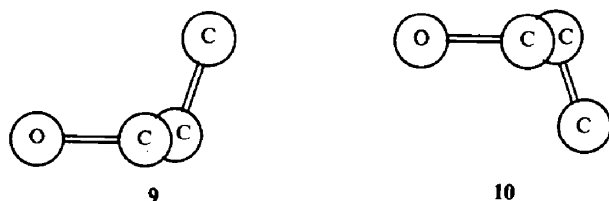
FIG. 5 Circular dichroism spectrum, conc  $6.41 \times 10^{-4}$  mole/l in dioxane.

\* A discussion of the conjugative possibilities between the CO group and the adjacent oxirane ring and of the importance of the orthogonal relationship of the plane of the oxirane ring and the plane of the  $\pi$  orbitals of the CO group to a third plane in the maximization of this effect may be found in Ref 11. † The same group relationship and its effect on the ORD characteristics of certain examples has been discussed.<sup>12</sup> ‡ A more recent consideration of the same relationship between the rather analogous cyclopropane ring and an adjacent CO group as it affects the UV absorption spectrum has been presented.<sup>13</sup>

† There is evidence that in uncomplicated cyclohexenones with non-planar chromophores the intensity of the UV absorption is diminished (as in this case) while the wave-length of the absorption maximum is the same as in related planar cases.<sup>14</sup> The red shift in the wave-length of the terremutin chromophore seems, then, to be due to the influence of the epoxide group.

‡ We thank Dr. N. Mitchell of the Cary Instrument Corporation for this spectrum. † We are particularly grateful to Dr. G. G. DeAngelis of these laboratories for putting us in touch with Dr. Mitchell and for a discussion of the interpretation of the spectrum.

causes a negative dichroism at  $318\text{ m}\mu$  ( $\epsilon = -5400^\circ$ ) and the  $n \rightarrow \pi^*$  absorption creates a band of low ellipticity ( $\epsilon = -1400^\circ$ ) at  $265\text{ m}\mu$ . It has been proposed<sup>15</sup> in a consideration of the conformation of cyclohexenones as related to ORD properties that in transoid skew cyclohexenones a double bond situated non-planar to the CO group and arranged as in **9** rather than as in **10** gives rise to a negative Cotton



effect for the  $n \rightarrow \pi^*$  transition (K-band) of  $\alpha,\beta$ -unsaturated ketones. By this rule conformer **5a** of terremutin would generate a negative Cotton effect at about  $250\text{ m}\mu$ . As noted above conformer **5a** also allows the maximum resonance interaction between the  $\pi$ -electrons of the CO group and the delocalized electrons of the oxirane ring.

An "inverse octant rule" has been proposed for  $\alpha,\beta$ -epoxy- and  $\alpha,\beta$ -cyclopropyl ketones which has been used successfully to predict the absolute configurations of a number of compounds.<sup>16</sup> It states that cyclohexanones with oxirane rings in the upper left or lower right quadrants cause negative Cotton effects for the  $n \rightarrow \pi^*$  transition. The octant projection of terremutin is shown in Fig. 6. It is obvious that the epoxide ring and the C-3 to C-4 double bond both lie in negative octants.

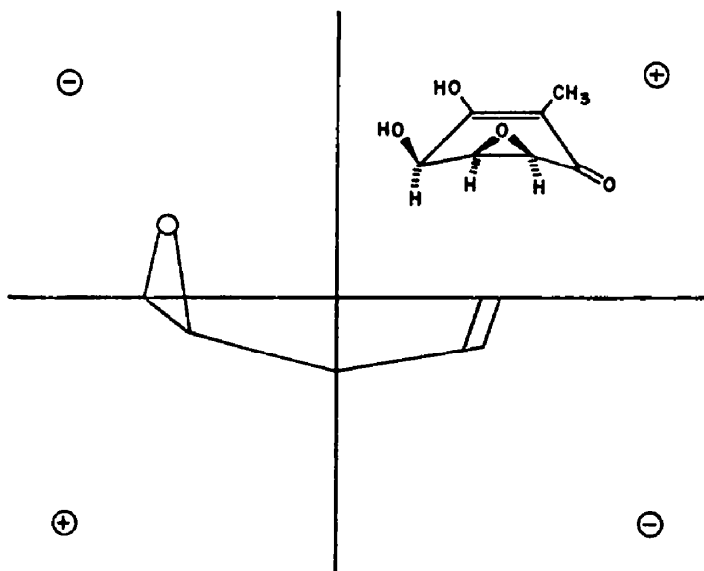
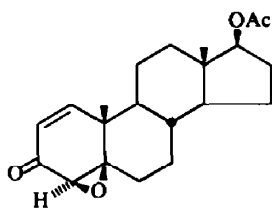


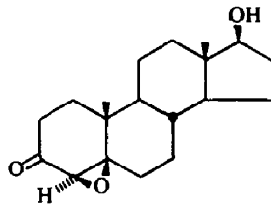
FIG. 6 Octant projection of terremutin.



Precedents exist for applicability of the inverse octant rule to a case such as terremutin, containing both an oxirane ring and a double bond. For example, it has been pointed out<sup>17</sup> that both 4 $\beta$ ,5 $\beta$ -oxido- $\Delta^1$ -pregnen-17 $\beta$ -ol-3-one acetate **11** and 4 $\beta$ ,5-epoxy-5 $\beta$ -androstane-3-one-17 $\beta$ -ol **12** showed the predicted *positive* Cotton effect

**11**

$$\Delta\epsilon = +5.92 (340 \text{ m}\mu)$$

**12**

$$\Delta\epsilon = +2.55 (340 \text{ m}\mu)$$

at about 340  $\text{m}\mu$ . Thus the occurrence of both a double bond and an epoxide ring adjacent to the carbonyl group apparently does not in this regard cause any unusual cross-conjugative aberrations.

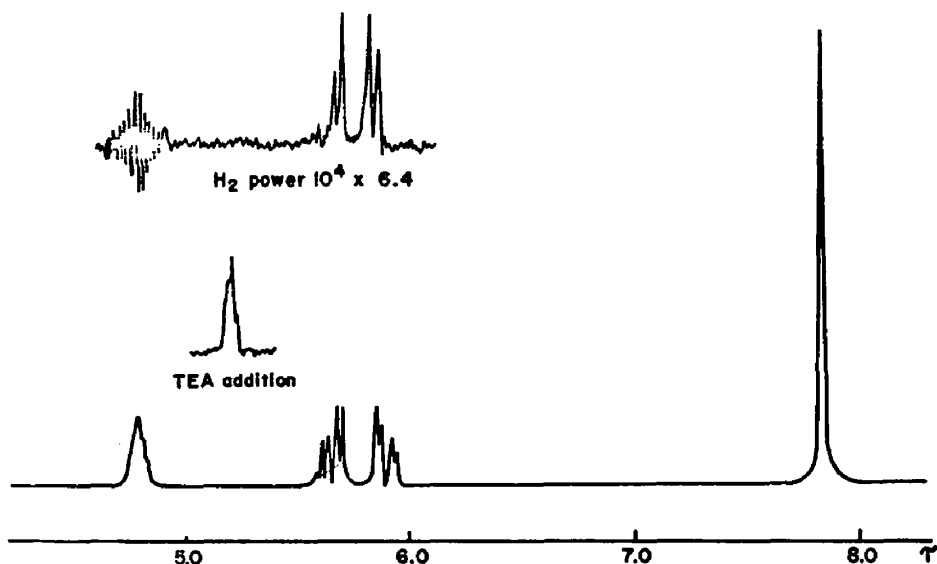


Fig. 7 Nuclear magnetic resonance spectrum of terremutin (20% in deuterium oxide).

Since the Cotton effect at 318  $\text{m}\mu$  is negative in terremutin, the oxirane ring is assigned the configuration shown in **5a**. It is, then, the mirror image of the epoxydon oxirane ring, and diastereoisomer **6** can be ruled out.

The configuration at C-5 was assigned on the basis of nuclear magnetic resonance studies. For this purpose the pertinent features of the spectrum of terremutin in  $\text{D}_2\text{O}$  shown in Fig. 7 are the C-5 proton signal centered at  $\tau$  4.8 and the pair of multiplets

with centers at  $\tau$  5.6 and  $\tau$  5.9. Addition of a few drops of triethylamine to the aqueous solution caused dissociation of the hydroxyl proton at C-4, and shielding by the oxygen anion so formed moved the C-5 proton signal upfield to  $\tau$  5.2, thus removing it from interference by the strong water signal and at the same time accentuating its fine structure so that the multiplet character became clearly revealed as shown in the inset. (The other signals were little affected.) Close inspection of the downfield signal indicated a triplet or quartet,  $J = \sim 1$  Hz, so that there must have been light coupling to the C-6 and C-1 protons. This apparent coupling was confirmed by double resonance experiments (spin-spin decoupling).<sup>\*</sup> Irradiation of the C-5 proton signal caused collapse of the C-6 to C-1 multiplet to a classic AB quartet pattern again shown by inset. Analysis of this quartet gave a coupling constant  $J = 4$  Hz, a reasonable figure for the structure proposed.<sup>†</sup> Irradiation at the upfield multiplet caused collapse of the adjacent multiplet to a simple pair, confirming the coupling of the vicinal protons of the epoxide group, and at the same time simplifying the subtle multiplet nature of the C-5 proton signal, thus confirming the long-range coupling between C-5 and C-1.

Inspection of a Dreiding model of conformer **5a**, already indicated as probable by the CD results, shows excellent agreement with the NMR measurements. The small coupling constant of about 1 Hz between the C-5 proton and the C-6 and C-1 protons indicates by application of the Karplus equation<sup>19</sup>: ‡ an angle of 60–70° between them. The model indicates that in conformer **5a** the angle between the proton at C-5 and either of the epoxide protons is indeed within this range, while in conformer **5b** the angles between all three protons would approach zero. Of course no long range coupling of the sort observed would be expected either in diastereoisomers **7** or **8**.

There remained some curiosity concerning the nature of the yellow crystalline pyridine "complex" with terremutin. The 20  $\mu$  blue shift in the UV absorption  $\lambda_{\max}$  relative to that of terremutin noted earlier was accompanied by an intensification of the extinction coefficient. ORD power was retained, but was different from that of terremutin anion. It was not possible to regenerate terremutin from the compound by simple means such as dissolution in water (yellow solution) or by addition of acid or base at ambient temperatures.

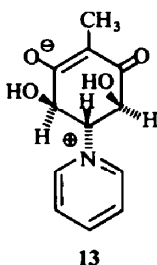
Using deuterated pyridine the compound was prepared, and the NMR spectrum in D<sub>2</sub>O obtained (cf. Fig. 8). It was apparent that the C-3 Me group signal was intact as was the C-5 proton signal, which appeared at  $\tau$  5.2 as in the triethylamine-generated anion, but with no fine-structure. This spectrum seems consistent with the structure **13** as well as with the other physical properties of the molecule. The slow formation of the crystalline substance from pyridine could indicate initial attack of the pyridine nitrogen at C-2 followed by rearrangement to **13**. In this form, the epoxide bond is opened correctly to yield the tetraacetate in fact obtained, although actually the oxide bond could be reformed intermediate to the tetraacetylation. Since the

We thank R. J. Hickey of our physical measurements laboratory for taking great pains with these experiments.

† For example, in styrene oxide the *cis*-vicinal protons of the epoxide ring have a  $J = 4$  Hz.<sup>18</sup>

‡ It is noteworthy that Dr. Karplus has since cautioned that his relationship of coupling constants to dihedral angles was derived only for uncomplicated cyclohexane rings, however it has been most useful in many empirical and non-quantitative applications to more complex structures. Certainly in the present instance the fit is good.

existing data on 13 did not seem to threaten the foregoing constitutional argument for terremutin, its structure was not pursued farther.



Terremutin, then, has been related structurally to terreic acid and to epoxydon. Many toluquinonoid substances are produced by molds.<sup>20</sup> We are not concerned here to any extent with the mode of biosynthesis of terremutin by *Aspergillus terreus*.

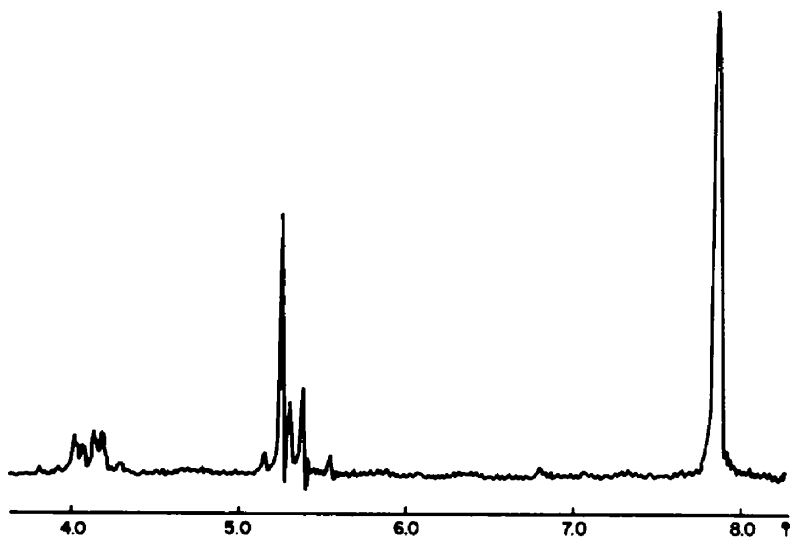


FIG. 8 NMR spectrum, terremutin-deuterated pyridine complex in  $D_2O$ .

The hydroquinones are known generally to be biosynthetic precursors of the quinones. It would be tempting to speculate that the mutation had interrupted an enzymic process completing the oxidation of the hydroquinonoid form of terreic acid to the quinoid form. It has been reported,<sup>21</sup> however, that the oxidative activity of the medium of surface cultures of *Aspergillus fumigatus*, previously ascribed to the action of exocellular phenolases, actually was due to autoxidation by the toluquinones already present. The rate of autoxidation of a hydroquinone was exponentially related to the energy of the highest occupied molecular orbital of the hydroquinone, enabling an estimation of the non-enzymic oxidation rates of the hydroquinone forms of all of the toluquinone pigments produced by this mold. Comparisons of these rates with that of

the natural process together with other evidence indicated that the rate of quinone formation was non-enzymic and related to the physical diffusion of oxygen in the medium.

## EXPERIMENTAL

M.p.s were taken on a calibrated Kofler hot-stage. NMR spectra were measured on a Varian Model A-60 machine. UV absorption spectra were taken on a Cary Model 13 recording spectrophotometer and IR spectra on a Perkin-Elmer Model 21 recording spectrophotometer. The mass spectrograph used was a Hitachi RMU-6E. A Rudolph photoelectric polarimeter, Model 200 was used; the analyses were carried out in our own physical measurements laboratory or by the Alfred Bernhardt Microanalytical Laboratory, Mühlheim, West Germany.

**Terremutin (1).** Terremutin was purified by several evaporative crystallizations from ether or AcOEt. Solvent removal was accomplished by pumping at 0.1 mm for 30 hr without heat or desiccant. If water was present a hydrate was obtained. Pure material melted at 144–146° (dec. much gas),  $[\alpha]_D^{27.5} - 269^\circ$  (c 1 in MeOH). The UV absorption characteristics were described above. The IR spectrum (KBr) had peaks at: 3.04 s, 3.24 s, 6.08 s, 6.14 s, 7.17 s, 7.46 m, 7.82 m, 7.79 m, 8.13 m, 8.89 m, 9.15 m, 9.64 s, 9.85 s, 11.11 m, 11.56 s, 12.20 s, 12.73 m, 14.14  $\mu$ . (Found: C, 54.25; H, 5.23. Calc. for  $C_7H_8O_4$ : C, 53.84; H, 5.16%).

Mol wt (mass spectrum) was 156, Calc. 156; neutral equivt: Calc. 156; Found 156; pH  $\frac{1}{2} = 5.25$ . MeO—negative. C-Me (Kuhn-Roth): 1. Active hydrogen (Zerewitnov with MeMgI in pyridine): 3. The NMR signals in  $D_2O$  occurred at  $\tau$  7.8 (s, 3H), 5.9 (m, 1H), 5.6 (m, 1H), 4.8 (s, 1H). When 2 drops of  $Et_3N$  were added to the soln, the singlet at  $\tau$  4.8 moved to  $\tau$  5.2 and developed more clearly defined multiplet character, the other signals remaining nearly unchanged.

**Terremutin-pyridine compound 13.** A 200 mg sample of terremutin was dissolved in 4 ml dry pyridine. A yellow color developed, and after 60 hr the bright yellow crystals which had separated were removed by filtration. Concentration of the filtrate caused more material to separate, total wt 215 mg crude, m.p. 185–190°. Recrystallization from butanol-dioxan-water gave pure yellow platelets, m.p. 182–183.5° (dec. gas). (Found: N, 6.2. Calc. for  $C_{12}H_{13}O_4N$ : N, 6.3%).  $[\alpha]_D^{24.5} - 174^\circ$  (c 1 in MeOH),  $[\alpha]_D^{24.5} - 285^\circ$  (c 1 in MeOH to which a few drops of  $Et_3N$  had been added);  $[\alpha]_D^{24.5} - 106^\circ$  (c 1 in MeOH 0.1N in HCl)  $[\alpha]_D^{24.5} + 14^\circ$  (c 1 in MeOH 0.1N in HCl after standing 14 hr). When the compound was prepared using deuterated pyridine, the NMR signals in  $D_2O$  occurred at  $\tau$  7.8 (s, 3H), 5.2 (s, 1H) a multiplet centered at 5.4 (1H) and a multiplet centered at 4.1 (1H).

The UV absorption spectrum in MeOH has a single peak,  $\lambda_{max}$  291  $m\mu$ ,  $\epsilon$  28,610. In MeOH, 0.01N in HCl,  $\lambda_{max}$  261  $m\mu$ ,  $\epsilon$  21,020 was obtained. In MeOH, 0.01N in NaOH, the  $\lambda_{max}$  was at 292  $m\mu$ ,  $\epsilon$  27,100. The IR spectrum showed bands at: 2.88, 3.24, 6.10, 6.19, 6.50, 6.70, 7.19, 7.56, 7.83, 8.10, 8.38, 8.86, 9.00, 9.25, 9.47, 9.93, 10.94, 11.40, 12.00, 12.65, 13.06 and 14.43  $\mu$ .

The complex was recovered unchanged after dissolution in water. Terremutin could not be regenerated from it by addition of base or acid.

**Conversion of terremutin to 2,3,5,6-tetraacetoxytoluene (2).** To a mixture of 500 mg  $Ac_2O$  and 5 ml dry pyridine was added 100 mg terremutin. After 20 min an amber color developed. At the end of 26 hr this color had vanished, and the white crystals which were present were removed by filtration. A second crop was obtained by pouring the filtrate into excess water to give a total crude yield of 159 mg (76%), m.p. 182–190°. Recrystallization from EtOH yielded white needles, m.p. 197–198°. (Found: C, 55.90; H, 5.08. Calc. for  $C_{15}H_{16}O_8$ : C, 55.55; H, 4.97%. Mol wt: Calc. 324; Found: (Rast in camphor) 336; C—Me (Kuhn-Roth): Calc. for 5 C—Me, 23.1. Found: 23.6).

The UV spectrum in MeOH showed a low broad peak,  $\lambda_{max}$  268,  $\epsilon$  745. In acidic MeOH there was little difference. In MeOH 0.01N in NaOH the  $\lambda_{max}$  was 299  $m\mu$ ,  $\epsilon$  5350 with a prominent shoulder at 325  $m\mu$ . The IR absorption spectrum (KBr) had bands at: 5.66, 6.22, 6.80, 6.97, 7.30, 8.35, 9.07, 9.40, 9.71, 9.87, 10.82, 11.23 and 11.65  $\mu$ .

The NMR signals were at  $\tau$  7.98 (3H, aromatic—Me)  $\tau$  7.78 (6H) and  $\tau$  7.72 (6H) due to the two different pairs of —COMe groups and at  $\tau$  2.95 (1H, aromatic).

When 100 mg of terremutin was acetylated with a mixture of  $EtOAc$ — $Ac_2O$ —perchloric acid (4 g of 70% perchloric acid in 150 ml of  $EtOAc$  and 50 ml of  $Ac_2O$ ) and the mixture allowed to stand at ambient temps for 72 hr, the same tetraacetate was obtained in 45% yield. A similar experiment interrupted at 20 hr yielded 8 mg crude crystals, m.p. 96–102°. The UV absorption curve of this substance in MeOH 0.01N in HCl had a  $\lambda_{max}$  at 234  $m\mu$ ,  $\epsilon$  9890. The IR absorption spectrum (KBr) showed no OH band but had bands at:

5-59, 5-65, 5-82, 5-97, 6-97, 7-24, 7-57, 8-00, 8-16, 8-29, 8-50, 8-99, 9-14, 9-31, 9-61, 9-85, 10-95, 11-10, 11-49, 12-18 and 13-93  $\mu$ .

*Dehydrogenation of terremutin to terreic acid (3).* A 100 mg sample of terremutin was mixed intimately with 500 mg 20% Pd-C catalyst. The mixture was heated (oil bath) under  $N_2$  in a small sublimation apparatus, gradually raising the temp to 250°. The press was reduced to 0.1 mm, and 40 mg crude yellow sublimate collected on the cold-finger. Recrystallization from benzene gave 16 mg straw-colored plates, m.p. 127–128°. Mixed with an equal amount of authentic terreic acid the m.p. was 127–128°.

*Hydrogenation of terremutin.* A 15 mg sample of 10% PC-C catalyst in 5 ml analytical grade MeOH in a 50 ml hydrogenation flask with side-arm dropping funnel was stirred magnetically and pre-reduced. When no more  $H_2$  was absorbed, a 100 mg (0.64 mmole) sample of terremutin in 5 ml MeOH was added from the side-arm, and hydrogenation continued. After 3 hr  $H_2$  uptake had virtually ceased. The uptake was 29.9 ml (cor) while the theoretical volume for 2 moles  $H_2$  was 28.7 ml. After 5 hr the catalyst was removed by filtration, and the filtrate concentrated to 95 mg of a pale yellow oil. No UV chromophore remained.

*Acknowledgements*—Profitable discussions of this work were held with Professor E. J. Corey, Professor Hans Muxfeldt, Professor Daniel Kemp and Doctor Gerald DeAngelis. Mr. Robert Martingano was the technical assistant on the problem.

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