3'-O-Methyl-3,4',5,7-tetra-O-benzylquercetin (V, R = $C_6H_5CH_2$–, R_1 = Me).—The above product (0.5 g.) was methylated by refluxing with excess of methyl iodide and potassium carbonate in acetone in the usual way. The acetone solution was evaporated to a gum. This was treated with water and the colorless, undissolved solid was collected. Successively recrystallized from methanol and from benzene–hexane, 3'-O-methyl-3,4',5,7-tetra-O-benzylquercetin separated in colorless, hair-like needles, m.p. 126–127° (air-dried for several days).

Anal. Calcd. for $C_{44}H_{36}O_7$: C, 78.1; H, 5.37; 1 MeO–, 4.61. Found: C, 78.2; H, 5.34; MeO–, 4.48.

Isorhamnetin (V, R = H, R_1 = Me).—The above methyl ether (0.3 g.) was dissolved in a mixture of glacial acetic acid (10.0 ml.) and concentrated hydrochloric acid (5.0 ml.). The solution was heated on the steam-bath for 1.5 hours, cooled and diluted with water. The yellow solid (80 mg.) was collected and recrystallized from acetone–methanol. Isorhamnetin thus was obtained as yellow needles, m.p. 305–306°.

Anal. Calcd. for $C_{16}H_{12}O_7$: C, 60.7; H, 3.83; 1 MeO–, 9.87. Found: C, 60.7; H, 4.00; MeO–, 10.6.

The acetate of the product, prepared by heating it with acetic anhydride and sodium acetate, crystallized from acetone-methanol in colorless needles, m.p. 205°.

Anal. Calcd. for $C_{24}H_{20}O_{11}$: C, 59.5; H, 4.16; 1 MeO–, 6.44; 4 CH_3CO–, 35.5. Found: C, 59.9; H, 4.21; MeO–, 6.29; CH_3CO–, 35.2.

3,4',7-Tri-O-benzylquercetin.—Quercetin pentaacetate (2.0 g.), benzyl chloride (5.0 ml.) and anhydrous potassium carbonate (5.0 g.) were refluxed in acetone (undried) for 18 hours. The filtered acetone solution was concentrated to an oil which was dissolved in methanol (20 ml.). On standing a slightly yellow crystalline product (0.4 g.) separated. This was recrystallized from methanol–acetone and from benzene–hexane. 3,4',7-Tri-O-benzylquercetin 5-monoacetate (VI, R = CH_3CO–) separated in slightly yellow needles, m.p. 148–150°.

Anal. Calcd. for $C_{38}H_{30}O_8$: C, 74.2; H, 4.92. Found: C, 74.4; H, 5.14.

Acetylation of this monoacetate gave 3,4',7-tri-O-benzylquercetin diacetate. This crystallized from benzene–hexane in colorless needles, m.p. 161–163°.

Anal. Calcd. for $C_{40}H_{32}O_9$: C, 73.1; H, 4.92; 2 CH_3CO–, 14.0. Found: C, 73.0; H, 5.04; CH_3CO–, 12.2.

The monoacetate (0.1 g.) was methylated in dry acetone by refluxing with methyl iodide and potassium carbonate. The product was purified by recrystallization from benzene–hexane; 3'-O-methyl-3,4',7-tri-O-benzylquercetin monoacetate thus was obtained in colorless needles, m.p. 164–165°

Anal. Calcd. for $C_{39}H_{32}O_8$: C, 74.5; H, 5.14; 1 MeO–, 4.97. Found: C, 74.5; H, 5.38; MeO–, 5.11.

The monoacetate (50 mg.) was hydrolyzed in a mixture of warm methanol (3.0 ml.) and 10% aqueous sodium hydroxide (1.0 ml.). The solution was diluted with water and acidified. The solid product was recrystallized from acetone–methanol. Slightly yellow needles of 3,4',7-tri-O-benzylquercetin (VI, R = H) thus were obtained, m.p. 140°.

Anal. Calcd. for $C_{36}H_{28}O_7$: C, 75.5; H, 4.93. Found: C, 75.4; H, 4.93.

4',7-Di-O-benzylquercetin (VII).—7-O-Benzylquercetin tetraacetate (1.0 g.) was benzylated in dry methyl ethyl ketone as described previously. In this one experiment, however, the product was not 3,4',5,7-tetra-O-benzylquercetin monoacetate but a dibenzyl derivative. The product was crystallized successively from benzene–hexane and acetone–methanol. 4',7-Di-O-benzylquercetin triacetate thus was obtained as colorless needles, m.p. 152–153°.

Anal. Calcd. for $C_{35}H_{28}O_{10}$: C, 69.0; H, 4.64; 3 CH_3CO–, 21.2. Found: C, 69.0; H, 4.69; CH_3CO–, 20.3.

The triacetate was hydrolyzed in dilute methanolic sodium hydroxide, 4,7-di-O-benzylquercetin (VII) crystallized from acetone–methanol in brightly yellow needles, m.p. 179°, which gave an olive-green ferric reaction and did not reduce Tollens reagent immediately.

Anal. Calcd. for $C_{29}H_{22}O_7$: C, 72.2; H, 4.60. Found: C, 72.1; H, 4.60.

Ultraviolet Spectra.—All spectra were determined in absolute ethanol solutions on a Cary recording spectrophotometer.

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PASADENA, CALIF.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY]

The Structure of Terreic Acid

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Terreic acid, an antibiotic metabolite of the mold *Aspergillus terreus*, has been converted by treatment with a mixture of acetic anhydride, acetic acid and boron trifluoride into 2,3,4,5,6-pentaacetoxytoluene (III). The action of dilute base upon the antibiotic produced a mixture of quinones, from which 2,3,5,6-tetraacetoxytoluene (IV) was obtained after reductive acetylation. A comparison of the n.m.r. spectra of terreic acid and 2,3-epoxy-1,4-naphthoquinone, together with other physical data and the chemical transformation products, allows the formulation of terreic acid as 5,6-epoxy-3-hydroxytoluquinone (I).

The isolation of the antibiotic terreic acid from culture broths of *Aspergillus terreus* was reported by Abraham and Florey[2] in 1949. Recently Kaplan, Hooper and Heinemann[3] obtained from cultures of an *Aspergillus* species an antibiotic which was demonstrated[4] to be identical with terreic acid. Although the antibiotic showed *in vitro* activity against gram-positive and gram-negative bacteria and fungi, *in vivo* tests were unpromising.[3] From a chemical standpoint, however, terreic acid is of interest because it possesses a combination of properties unusual for compounds of comparable molecular size. In this paper evidence is presented for the formulation of terreic acid as 5,6-epoxy-3-hydroxytoluquinone (I).

Terreic acid, $C_7H_6O_4$, may be purified either by sublimation or crystallization to give pale yellow

(1) (a) National Institutes of Health Postdoctoral Fellow, 1956–1957; (b) Bristol Laboratories Fellow, 1953–1954.

(2) E. P. Abraham and H. W. Florey, "Antibiotics," H. W. Florey, *et al.*, eds. Oxford University Press, New York, N. Y., 1949, Vol. I, p. 337. The discovery that *Aspergillus terreus* produces an antibiotic substance was made by W. H. Wilkins and G. C. M. Harris, *Brit. J. Exp. Path.*, **23**, 166 (1942).

(3) M. A. Kaplan, I. R. Hooper and B. Heinemann, *Antibiotics & Chemotherapy*, **4**, 746 (1954).

(4) Comparisons were made by Dr. K. R. Henery-Logan of this laboratory with the last few existing milligrams of authentic terreic acid, which were very kindly supplied by Professor Sir Howard Florey of Oxford. Terreic acid from Bristol Laboratories had m.p. 127–127.5°, while that from Oxford had m.p. 120–121°, but a mixed m.p. of 126.5–127° indicated that the two samples were identical, as did comparisons of their infrared and ultraviolet spectra.

needles, m.p. 127–127.5°, which are irritating to the mucous membranes. The antibiotic is optically active, and the rotation varies considerably with the solvent. A Kuhn–Roth determination showed the presence of one C-methyl group. The acidity is due to an enolic hydroxyl group, since terreic acid (pK_a 4.5), which gives a red color with ferric chloride, is converted by diazomethane into a neutral monomethyl derivative, $C_8H_8O_4$, which gives a negative ferric chloride test.

The antibiotic reduces Fehling and Tollens solutions, consumes periodic acid, and gives a precipitate with 2,4-dinitrophenylhydrazine. Although moderately stable in acid solution (*p*H 2), terreic acid rapidly decomposes in alkali, with the generation of a second acidic group.[3]

In the infrared, terreic acid possesses strong, sharp peaks at 3300, 1690, 1655 and 1629 cm.$^{-1}$, compatible with the presence of an enolized 1,2,4-triketone system. The spectrum as a whole is very similar to that of 3,6-dihydroxytoluquinone (II).[5] The ultraviolet spectrum has maxima at 214 mμ (log ε 4.03) and 316 mμ (log ε 3.88). The latter band is shifted to lower wave lengths in acid solution (304 mμ in *N* hydrochloric acid) or by conversion of the antibiotic to the methyl ether (305 mμ).

Degradation products which indicated the presence of a six-membered carbon ring in terreic acid were obtained as follows. When the antibiotic was treated with a mixture of acetic acid and acetic anhydride containing boron trifluoride, a colorless, crystalline pentaacetate, $C_7H_3(OAc)_5$ (m.p. 177°), was obtained. The ultraviolet spectrum of the pentaacetate was typically benzenoid, having an $\epsilon_{max.}$ of 342 at 262 mμ and high end absorption. The infrared spectrum possessed a single, broad, intense peak in the carbonyl region at 1780 cm.$^{-1}$. The most reasonable structure for the acetate was the previously unknown 2,3,4,5,6-pentaacetoxytoluene (III).

Synthetic 2,3,4,5,6-pentaacetoxytoluene was prepared by reductive acetylation of 3,5,6-trihydroxytoluquinone (V) (available *via* a four-step synthesis from orcinol[6]) and shown to be identical with the pentaacetate from terreic acid.[7]

When the antibiotic was treated with *N* sodium hydroxide (2 minutes at 25°), a mixture of quinones was obtained. The presence of 3,6-dihydroxytoluquinone (II) seemed likely, and was confirmed by reductive acetylation of the mixture and isolation of 2,3,5,6-tetraacetoxytoluene (IV), identical with an authentic sample.[11]

The isolation of compounds containing benzenoid rings (III and IV) after treatment of terreic acid with both acidic and basic reagents constitutes good evidence for the presence of a six-membered carbon ring in the antibiotic. A bicyclic structure is necessary, however, to explain the optical activity. Taking into account the molecular formula, optical activity, enolic acidity, presence of a C-methyl group, the chemical transformation products and the infrared and ultraviolet spectra, 5,6-epoxy-3-hydroxytoluquinone (I) is proposed as the structure of terreic acid.

Further evidence for structure I was obtained from the nuclear magnetic resonance (n.m.r.) spectrum of terreic acid, and in addition by a comparison with the n.m.r. spectrum of 2,3-epoxy-1,4-naphthoquinone (VI) (*cf.* Table I). The spectrum of terreic acid has three peaks, attributable[12] to the hydrogen atoms on the methyl group, the epoxide ring and the acidic hydroxyl group (the proportionate magnitudes of which are *ca.* 3:2:1, respectively). The assignment of the peak at +30 c.p.s. in the spectrum of terreic acid to the hydrogen atoms on the epoxide ring was substantiated by examination of the n.m.r. spectrum of 2,3-epoxy-1,4-naphthoquinone (VI). In addition to a peak attributable to the hydrogen atoms on the benzene ring, there is a peak at +32 c.p.s., which could be due only to the hydrogens on the epoxide ring.

We are indebted to Bristol Laboratories of Syracuse, N. Y., for our supply of terreic acid, and for some financial aid.

Experimental[13]

Terreic Acid (I).—Terreic acid (Bristol Laboratories) crystallizes as pale yellow plates from benzene or as needles from benzene–ligroin, m.p. 127–127.5°, $[\alpha]^{27}D$ −12.6° (*c* 0.86 in 50% aqueous methanol), λ_{max} 214 mμ (log ε 4.03) and 316 mμ (log ε 3.88) in ethanol [reported[3] m.p. 127–127.5°, $[\alpha]^{22}D$ −16.6° (*c* 1 in chloroform), $[\alpha]^{22}D$ −28.6° (*c* 1 in methanol–benzene [1:1]), $[\alpha]^{22}D$ +74.3° (*c* 1 in *p*H 7 phosphate buffer), λ_{max} 213 mμ (log ε 4.01) and 316 mμ (log ε 3.79)]. The infrared spectrum (potassium bromide) had peaks at 3300(s), 1690(s), 1655(s), 1629(s), 1380(s), 1370(s), 1350(s), 1305(s), 1250(m), 1220(m), 1200(s), 1135(s), 1035(s), 990(w), 865(m), 836(w), 789(m), 760(s), 715(m-sh) and 700 cm.$^{-1}$(m).

Anal. Calcd. for $C_7H_6O_4$: C, 54.55; H, 3.93; 1 C-CH_3, 9.75; mol. wt., 154. Found: C, 54.87; H, 3.92; C-CH_3, 8.48; neut. equiv., 154; mol. wt., 134 (cryoscopic in dioxane).

Terreic Acid Methyl Ether.—To a cooled (*ca.* 5°) solution of 500 mg. (3.2 mmoles) of terreic acid in 40 ml. of benzene was added 25 ml. of a benzene solution of diazomethane (10 mg./ml.). The resulting solution was evaporated to 40 ml. on a steam-cone and extracted with 10% sodium bicarbonate until the aqueous extract was colorless. The organic layer was washed with water, dried (magnesium sulfate) and concentrated. The residual oil was distilled evaporatively at 80° (0.1 mm.) to give 240 mg. (44%) of colorless solid,

(5) *Cf.* Experimental section. There is an excellent correspondence of bands. In the double bond region the spectrum of the quinone possesses a single, diffuse band centered at about 1610 cm.$^{-1}$, while that of terreic acid has three sharp bands between 1700 and 1600 cm.$^{-1}$. Broad, intense absorption in two other areas of the quinone spectrum (1380–1300 and 1200–1170 cm.$^{-1}$) are found instead of the several sharp, intense peaks in the same regions of the terreic acid spectrum.

(6) V. Merz and G. Zetter, *Ber.*, **12**, 2044 (1879).

(7) Treatment of 3,6-dihydroxytoluquinone (II) with acetic anhydride and strong acid (Thiele reaction) appeared to be a simple way of preparing III. However, attempts to carry out the reaction using sulfuric acid,[8] boron trifluoride etherate[9] or perchloric acid[10] as catalysts failed. The mixture from the perchloric acid-catalyzed reaction was converted by reductive acetylation into 2,3,5,6-tetraacetoxytoluene (IV), a derivative of the starting material.

(8) J. Thiele and E. Winter, *Ann.*, **311**, 351 (1900); W. K. Anslow and H. Raistrick, *Biochem. J.*, **32**, 687 (1938).

(9) L. F. Fieser, THIS JOURNAL, **70**, 3165 (1948).

(10) H. Burton and P. F. G. Prail, *J. Chem. Soc.*, 755 (1952).

(11) 2,3,5,6-Tetraacetoxytoluene (IV) was isolated easily by virtue of its insolubility, and was not, of course, the only product. Another acetate, presumably 2,3,4,6-tetraacetoxytoluene, apparently was formed in greater yield than IV, but was not isolated in a pure state. It is unfortunate that lack of material prevented the repetition of this work on a larger scale.

(12) Professor John S. Waugh, private communication. We are indebted to Professor Waugh for his help with the n.m.r. spectra.

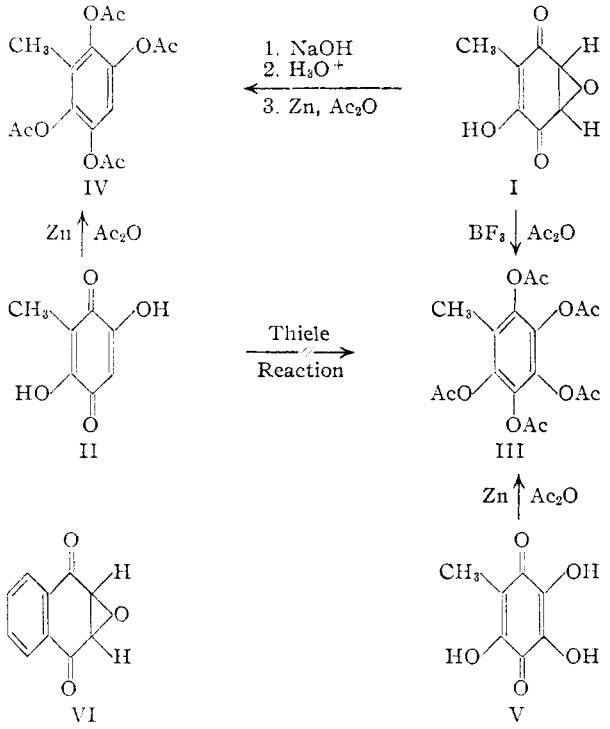
(13) All melting points are corrected. The analyses were by Dr. S. M. Nagy and his associates.

TABLE I

N.M.R. SPECTRA OF TERREIC ACID (I) AND 2,3-EPOXY-1,4-NAPHTHOQUINONE (VI)

The n.m.r. spectra were determined with *ca.* 5 and 2.5% solutions in deuteriochloroform at 40 mc./sec. and extrapolated to infinite dilution. Peaks were measured relative to the OH absorption of water, obtained by the use of internal water capillaries.

Terreic acid peak, c.p.s. (ratio)	Epoxynaphthoquinone peak, c.p.s. (ratio)	Assignment
+107 (3)	.....	Methyl hydrogens
+30 (2)	+32 (1)	Epoxy hydrogens
−93 (1)	.....	Hydroxyl hydrogens
.....	−126 (2)	Aromatic hydrogens



m.p. 41.8–42.8°, $[\alpha]^{25}D$ −66.4° (*c* 1.52 in chloroform), λ_{max} 305 mμ (log ε 3.74) in 95% ethanol.

Anal. Calcd. for $C_8H_8O_4$: C, 57.14; H, 4.79; OCH_3; 18.46; mol. wt., 168. Found: C, 57.27; H, 4.93; OCH_3, 18.67; mol. wt. (Rast in exaltone), 172 ± 10.

2,3,4,5,6-Pentaacetoxytoluene (III). A. From Terreic Acid.—Boron trifluoride was bubbled into a mixture of 7 ml. of acetic anhydride and 3 ml. of acetic acid until the temperature of the solution had risen to 55°. To 1.0 ml. of the above solution, 75.5 mg. of terreic acid was added, and the mixture was heated on a steam-bath for 20 min. The resulting solution was stored at room temperature for 24 hr., after which period 2 ml. of water and 3 ml. of ethyl acetate were added. The organic layer was washed with water and dried (magnesium sulfate). Evaporation gave an orange oil, which was dissolved in ethyl acetate and concentrated to 0.1 ml. Trituration with benzene gave 52.3 mg. of light tan crystals, m.p. 166–173°. Two recrystallizations from ethyl acetate–cyclohexane gave 32.9 mg. of colorless crystals of 2,3,4,5,6-pentaacetoxytoluene, m.p. 175.5–177.0°, λ_{max} 262 mμ (log ε 2.53) in ethanol. This material did not depress the m.p. of authentic pentaacetoxytoluene (*vide infra*), and the infrared and ultraviolet spectra of the two samples were the same.

Anal. Calcd. for $C_{17}H_{18}O_{10}$: C, 53.40; H, 4.75; acetyl (5), 56.30. Found: C, 53.50; H, 4.91; acetyl, 56.23.

B. From 3,5,6-Trihydroxytoluquinone (V).—Trinitroorcinol, prepared by the procedure of Stenhouse,[14] was converted into 3,5,6-trihydroxytoluquinone by the method of Merz and Zetter.[6] After the reduction of 3 g. of trinitroorcinol to triaminoörcinol, oxidation with ferric chloride gave 840 mg. of 2-imino-3-hydroxy-4,6-diaminotoluquinone (VII). The crude 3,5,6-trihydroxytoluquinone formed by acid hydrolysis of 400 mg. of VII (10 min. in boiling 5 *N* sulfuric acid)[15] was extracted into ethyl acetate, and the dried (sodium sulfate) solution was evaporated. The red-black residue was refluxed for 30 min. with 1 g. of zinc dust in acetic anhydride–acetic acid (7:3). The zinc was removed and the filtrate extracted with 10% sodium chloride solution, *N* potassium bicarbonate solution and dried over sodium sulfate. After evaporation to dryness, the residue crystallized from benzene to give 113 mg. of 2,3,4,5,6-pentaacetoxytoluene, m.p. 174–176°. Recrystallization from ethyl acetate–cyclohexane and from ethanol gave material which had m.p. 175.5–177.0°, λ_{max} 262 mμ (log ε 2.53).

2,3,5,6-Tetraacetoxytoluene (IV).—A solution of 150 mg. of terreic acid in 3 ml. of *N* sodium hydroxide was allowed to stand at room temperature for 2 min. After acidification with 3 *N* hydrochloric acid the reddish-brown solution was extracted with ethyl acetate, and the dried extract evaporated. The residue (consisting of a mixture of quinones)[16] was reductively acetylated with 5 ml. of acetic anhydride and 1 g. of zinc dust to give, after crystallization from ethanol–water, 14.6 mg. of colorless crystals, m.p. 175–190°. After two further recrystallizations, fine needles of 2,3,5,6-tetraacetoxytoluene, m.p. 198.5–200.0°, were obtained. A mixed m.p. with authentic 2,3,5,6-tetraacetoxytoluene,[17] m.p. 200.0–203.5°, was 199.5–203.0°.

1,4-Naphthoquinone-2,3-epoxide (VI).—1,4-Naphthoquinone-2,3-epoxide was prepared by Fieser's modification[9] of Zincke's method,[18] m.p. 133–135° (reported m.p. 132–133° [Fieser]; and 136° [Zincke]).

3,6-Dihydroxytoluquinone (II).—The infrared spectrum (potassium bromide) of 3,6-dihydroxytoluquinone[19] was very similar to that of terreic acid, having peaks at 3300(s), 1610(s), 1380–1300(s), 1240(m), 1210–1170(s), 1130(s-sh), 1050(s), 989(w), 859(s), 820(w), 783(w-sh), 765(s), 735(w) and 700 $cm.^{-1}$(s).

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(14) J. Stenhouse, *Chem. News*, **23**, 193 (1871).

(15) Method used by W. K. Anslow and H. Raistrick, *J. Chem. Soc.*, 1446 (1939), for the hydrolysis of aminoquinones to hydroxyquinones.

(16) A small quantity of an orange quinone (more volatile) was obtained by sublimation, but was not well separated in this manner from a purple quinone (less volatile). Like 3,6-dihydroxytoluquinone, m.p. 177°, the orange quinone, m.p. *ca.* 179°, gave a purple color with concentrated sulfuric acid and with dilute sodium hydroxide. The purple quinone, presumably the unknown 3,5-dihydroxytoluquinone, gave a green color with both concentrated sulfuric acid and dilute sodium hydroxide.

(17) F. Fichter, *Ann.*, **361**, 400 (1908). The reported m.p. is 198° The 2,3,5,6-tetraacetoxytoluene had λ_{max} 268 mμ (log ε 678).

(18) T. Zincke, *Ber.*, **25**, 3599 (1892).

(19) T. Zincke, *ibid.*, **16**, 1558 (1883); *cf.* also ref. 15.