ring at 50° for 1 hr and cooled in an ice bath. The solid was collected by filtration, washed with H_2O, and recrystallized. See Table I.

3,4-Dichlorophenyl 2-Furyl Ketone (Ia). 2-Furoyl chloride (195 g, 1.5 mol) was added dropwise to a mixture of 200 g (1.5 mol) of anhydrous $AlCl_3$ and 500 ml of ethylene chloride over 15 min with stirring. *o*-Dichlorobenzene (220 g, 1.5 mol) was added dropwise over 20 min. The mixture was heated on a steam bath for 20 hr and poured into a mixture of concentrated HCl and ice. The organic layer was separated, and the aqueous layer was extracted with $CHCl_3$. The combined organic layers were washed with 5% $NaHCO_3$ solution and H_2O and dried (K_2CO_3). The solvent was removed by distillation under reduced pressure, and the residual oil was distilled to give 51 g (15%) of Ia: bp 182° (1.0 mm); ir (Nujol) 1640 cm^{-1} (C=O); nmr ($CDCl_3$) δ 6.54 (dd, 1, J = 1.8 and 3.5 Hz, 4-furyl proton), 7.24 (d, 1, J = 3.8 Hz, 3-furyl proton), 7.49 (d, 1, J = 8.2 Hz, 5-phenyl proton), 7.66 (dd, 1, J = 0.9 and 1.7 Hz, 5-furyl proton), 7.81 (dd, 1, J = 2.0 and 8.5 Hz, 6-phenyl proton), and 8.06 ppm (d, 1, J = 2.0 Hz, 2-phenyl proton). *Anal.* ($C_{11}H_6Cl_2O_2$) C, H.

3-Fluoro-4-methoxyphenyl 2-Furyl Ketone (Ib). Anhydrous $AlCl_3$ (117 g, 0.88 mol) was added in portions to a mixture of 104 g (0.80 mol) of 2-furoyl chloride, 100 g (0.80 mol) of *o*-fluoroanisole, and 500 ml of CS_2 over 1.5 hr with stirring and cooling. The mixture was stirred for 3.5 hr at room temperature, heated under reflux for 2.5 hr, allowed to stand overnight, and poured into a mixture of ice and H_2O. The organic layer was separated, and the aqueous layer was extracted with $CHCl_3$. The combined organic layers were washed twice with 10% Na_2CO_3 solution and once with H_2O and dried ($MgSO_4$). The solvent was removed by distillation under reduced pressure, and the residual solid was recrystallized from MeOH to give 148 g (84%) of Ib. Recrystallization again from MeOH gave an analytical sample: mp 80.5–81°; ir (Nujol) 1645 cm^{-1} (C=O). *Anal.* ($C_{12}H_9FO_3$) C, H.

3-Fluoro-4-methoxybenzoic Acid. A solution of 27 g of $KMnO_4$ in 250 ml of hot H_2O was added slowly to a suspension of 13 g (0.06 mol) of Ib in 100 ml of hot H_2O containing 0.2 g of KOH. The mixture was heated on a steam bath for 45 min, and the MnO_2 was removed by filtration. The filtrate was made acidic with concentrated H_2SO_4, and the white solid was collected by filtration to give 3 g (30%), mp 212–214° (lit.[7] mp 211.6–212.8°).

Aryl 5-Nitro-2-furyl Ketone Oximes (10–12). A mixture of 0.25 mol of **1**, **2**, or **3**, 1.0 mol of $NH_2OH \cdot HCl$, 1.0 mol of anhydrous NaOAc, and 2 l. of 75% aqueous EtOH was heated under reflux for 5 hr and cooled. The solid was collected by filtration and dried. See Table I for yields. Analytical samples were prepared by recrystallization from the solvents indicated in Table I.

5-Nitro-2-furyl Phenyl Ketone *O*-Acetyloxime (14). A mixture of 71 g (0.30 mol) of **10**, 61 ml (0.60 mol) of Ac_2O, and 700 ml of *p*-dioxane was heated under reflux for 19 hr. After cooling, the mixture was poured into 2 l. of cold H_2O, and the solid was collected by filtration. Recrystallization from EtOAc gave 41 g of **14**.

5-Nitro-2-furyl Phenyl Ketone *O*-Benzoyloxime (15). Benzoyl chloride (1.8 g, 0.013 mol) was added dropwise to a solution of 2.3 g (0.01 mol) of **10** in 15 ml of pyridine with stirring. The solution was stirred at room temperature for 6 hr and allowed to stand overnight. Water (30 ml) was added, and the solid was collected by filtration and washed with H_2O. Recrystallization from $MeNO_2$ twice gave 1.2 g of **15**.

5-Nitro-2-furyl Phenyl Ketone *O*-Methyloxime (13), Semicarbazone (16), Thiosemicarbazone (17), and Aminohydantoin (18). Concentrated HCl was added dropwise to a hot solution (0.20 mol) of **1** and (0.24 mol) of methoxyamine hydrochloride, semicarbazide hydrochloride, thiosemicarbazide, or 1-aminohydantoin in 600 ml of 80% aqueous EtOH to adjust the pH to 3. The solution was heated under reflux for 4 hr and cooled in an ice bath. The solid was collected by filtration and washed with cold 80% aqueous EtOH. See Table I for yields. Analytical samples were prepared by recrystallization from the solvents indicated in Table I.

Kinetic Fungicidal Test. Hog gastric mucin (Wilson) dissolved in Sabouraud's liquid medium (BBL) at a 5% concentration (w/v) was sterilized by autoclaving. To the cooled medium, sufficient sterile bovine serum was added to obtain a 10% concentration, v/v. Test compounds were dissolved in *N,N*-dimethylacetamide (0.2–0.5 ml), and sufficient medium was added to obtain the requisite test concentrations. The exact amount of solvent used was added to a separate flask for control.

The 24-hr agar slants of the respective yeast strain were harvested in saline. Cell concentrations were determined by a haemocytometer count or Klett-Summerson colorimeter readings plus previously prepared standard growth curves. Sufficient cells were added to the test medium to give 4×10^5 cells/ml. Aliquots were obtained from the flask samples, serially diluted in saline, and plated in medium to obtain a viable count. Samples were taken at 0, 2, 4, 7, 24, and 72 hr. The data obtained would indicate whether the test compound was active, inactive, fungistatic, or fungicidal and the rate of fungicidal activity if present.

References

(1) A. P. Dunlop and F. N. Peters, "The Furans," Reinhold, New York, N. Y., 1953, p 156.
(2) T. Tamura, E. Niwa, H. Tanaka, and H. Aoki, *Nippon Nogei Kagaku Kaishi*, **41** (2), 64 (1967); *Chem. Abstr.*, **67**, 73458y (1967).
(3) G. A. Olah, Ed., "Friedel-Crafts and Related Reactions," Vol. III, Part 1, Interscience, New York, N. Y., 1964, p 46.
(4) Reference 3, p 50.
(5) Ng. Ph. Buu-Hoi, Ng. D. Xuong, and D. Lavit, *J. Org. Chem.*, **18**, 910 (1953).
(6) J. English, Jr., J. F. Mead, and C. Niemann, *J. Amer. Chem. Soc.*, **62**, 350 (1940).
(7) J. T. Minor and C. A. Vanderwerf, *J. Org. Chem.*, **17**, 1425 (1952).
(8) D. C. Grove and W. A. Randall, "Assay Methods of Antibiotics: A Laboratory Manual," Medical Encyclopedia, New York, N. Y., 1955.

Synthesis of Pyrimido[4,5-*b*][1,4]-7-hydrothiazines Related to 7,8-Dihydropteridines of Biological Importance†,‡

Young-Ho Kim and Henry G. Mautner*

Department of Biochemistry and Pharmacology, Tufts University School of Medicine, Boston, Massachusetts 02111.

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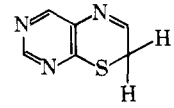
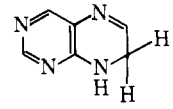
7,8-Dihydrofolic acid and related 7,8-dihydropteridines are key metabolic intermediates in a variety of biological reactions, while antagonists of such compounds have been found to have useful antineoplastic[1,2] and immunosuppressant activities.[3] It has been reported that substituted 7,8-dihydropteridine analogs of antagonists of folic and pteroic acid may be more potent inhibitors of *Streptococcus faecalis* and of *Pediococcus cerevisiae* than their nonreduced pteridine analogs.[4,5]

Since most of the useful biological properties of substituted pteridines are related to their abilities to form dihydro and tetrahydro derivatives, it is unfortunate that the high reactivity of these reduced forms both to oxidative degradation and photoreactions makes it difficult to work with them.[6] Work with reduced analogs of folic acid, pteroic acid, and related derivatives is also complicated by the extremely low solubilities of these compounds at physiological pH, presumably due to hydrogen bonds formed by their ring nitrogens. It was hoped that replacement of the 8-nitrogen of reduced pteridines with sulfur would provide compounds with greater stabilities and better solubility characteristics than those of their analogous 7,8-dihydropteridine derivatives. While several members of this ring system have been prepared previously,[7-12] none of these have been related to biologically active dihydropteridines.



pyrimido[4,5-*b*][1,4]-7-hydrothiazine



7,8-dihydropteridine

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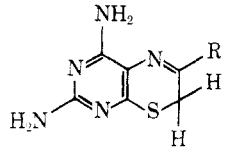
‡The research was presented at the 166th National Meeting of the American Chemical Society, Chicago, Ill., Aug 1973, MEDI 006.

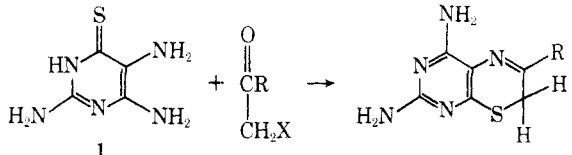
Table I. Inhibition of *Streptococcus faecalis* (ATCC-8043)



R	Concn, mγ/ml[a]
$-C_6H_5$	400
-COOH	>2000
$-COOC_2H_5$	>2000
$-(CH_2)_2NHC_6H_4$-*p*-COOH	40
$-(CH_2)_2NHC_6H_4$-*p*-COOEt	400

[a]Concentration necessary to induce 50% growth inhibition (folic acid 1 mγ/ml).

The key intermediate in the synthesis of pyrimido[4,5-*b*][1,4]-7-hydrothiazines, 2,5,6-triamino-4-thiopyrimidine (1), has been prepared by Elion, Lange, and Hitchings[13] using a reaction involving the coupling of 2,6-diamino-4-thiopyrimidine with *p*-chlorophenyldiazonium chloride, followed by reduction. 2,5,6-Triamino-4-thiopyrimidine can be obtained more simply from 2,5,6-triamino-4-hydroxypyrimidine by a direct thiation reaction.[14] The yield of the thiation could be improved by the use of collidine as solvent. Reaction of the triaminothiopyrimidine with α-halo ketones in the presence of sodium acetate yields substituted pyrimido[4,5-*b*][1,4]-7-hydrothiazines.



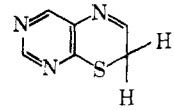
1

2, R = $-C_6H_5$
3, R = -COOEt
4, R = -COOH
5, R = $-(CH_2)_2NHC_6H_4$-*p*-COOEt
6, R = $-(CH_2)_2NHC_6H_4$-*p*-COOH

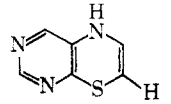
The compounds **2** and **3** were prepared from commercially available α-halo ketones and the thiopyrimidine **1**. Compound **4** was obtained by the alkaline hydrolysis of **3**. The precursors of **5** and **6**, including the new carboxylic acids **8** and **10**, were synthesized by the procedure of Kim, *et al.*[15]

While the reaction sequence used results in the formation of aminopyrimidohydrothiazines *via* S-alkylation[7,10,11] rather than, as in the condensation with α,β-dicarbonyl compounds, in the formation of thiopteridines, it still remained to establish which tautomers are formed.

Nuclear magnetic resonance spectroscopy of the compounds synthesized showed two protons at C_7 appearing as a singlet between 3.51 to 3.98 ppm and no evidence for a vinyl group. Thus, these compounds belong to the pyrimido[4,5-*b*]-7-hydrothiazine and not to the pyrimido[4,5-*b*]-5-hydrothiazine series.



pyrimido[4,5-*b*]-7-hydrothiazine



pyrimido[4,5-*b*]-5-hydrothiazine

All the products were soluble in dimethyl sulfoxide. Compounds **2**, **3**, and **5** were also soluble in ethanol, acetonitrile, and ethyl acetate. In all cases, these products were considerably more soluble than their pteridine analogs.

The antimicrobial activity of the various products is summarized in Table I. Only compound **4**, an analog of 4-aminohomopteroic acid, had significant inhibitory activity.

Experimental Section§

2,5,6-Triamino-4-thio-1*H*-pyrimidine Dihydrochloride (1). At 60° P_2S_5 (10 g) was added to collidine (175 ml) in portions with stirring. The temperature was raised to 90° and 2,5,6-triamino-4-oxo-1*H*-pyrimidine dihydrochloride (5 g, 0.023 mol), obtained from its sulfate, was added in small portions. The resulting heterogeneous mixture was heated with stirring at 165–170° for 17 hr. The two-phase mixture was then cooled to room temperature with stirring and the excess collidine was removed by decantation. The residue was quickly covered with anhydrous Et_2O and the cake broken up. The solid was washed with Et_2O (2 × 30 ml) to remove any free collidine. Hydrochloric acid (6 *N*, 150 ml) was gradually added to the solid with stirring at 0° within 10 min and then the resultant mixture was stirred at room temperature for 40 min. A negligible amount of insoluble material was removed by filtration and filtrate was evaporated to dryness *in vacuo* at 35°. The solid residue was thoroughly triturated with MeOH to remove collidine hydrochloride to give 3.2 g (62%) of desired product: mp >360°; slowly dec >250°; λ_{max} (0.1 *N* HCl) 310 nm (ε 22.8 × 10^3).[13]

2,4-Diamino-6-phenylpyrimido[4,5-*b*][1,4]-7-hydrothiazine (2). To a solution of **1** (1.1 g, 0.005 mol) and AcONa (2 g, 0.024 mol) in H_2O–EtOH (150 ml, 1:1) was added phenacyl bromide (1.0 g, 0.005 mol). The homogeneous reaction mixture was stirred at room temperature for 4.5 hr and was treated with charcoal. Ethanol was removed *in vacuo* at 50°. The yellow precipitate was collected by filtration and washed with H_2O to give 0.88 g (68%) of crude product, mp 190–192°. Recrystallization from AcOEt gave an analytically pure product: mp 200–202°; nmr (TFA) 3.98 ppm (s); λ_{max} nm (ε × 10^3) (95% EtOH) 240 (18.3), 274 (17.8), 381 (12.9). *Anal.* ($C_{12}H_{11}N_5S$) C, H, N.

2,4-Diamino-6-carbethoxypyrimido[4,5-*b*][1,4]-7-hydrothiazine (3). The reaction was carried out over 2 hr at room temperature, under the conditions described in **2** in 36% yield: mp 194–197°; dec >180°. The analytical sample was recrystallized from AcOEt: mp 209–211°; nmr 3.91 ppm; λ_{max} nm (ε × 10^3) (95% EtOH) 219 (20.4), 282 (15.6), 393 (11.4). *Anal.* ($C_9H_{11}N_5O_2S$) C, H, N.

2,4-Diamino-6-carboxypyrimido[4,5-*b*][1,4]-7-hydrothiazine (4). A heterogeneous mixture of **3** (200 mg) in 0.2 *N* NaOH (20 ml) was heated with stirring at 95° under nitrogen for 1 hr. Within 15 min the mixture became homogeneous. The resultant solution was treated with charcoal. The pH was carefully adjusted to 3.5 at 0° with 0.1 *N* HCl. The precipitated product was collected by centrifugation and washed twice with H_2O, with CH_3CN, and then with Et_2O to give the hydrolyzed product (100 mg, 57%): dec >230°; mp 258–260°; nmr (1 *M* Na_2CO_3 in D_2O) 3.81 ppm; λ_{max} nm (ε × 10^3) (0.01 *N* NaOH) 223 (19.2), 267 (14.1), 367 (8.2). *Anal.* ($C_7H_7N_5O_2S \cdot H_2O$) C, H, N.

2,4-Diamino-6-[2-*N*-(*p*-carbethoxyphenyl)amino]ethylpyrimido[4,5-*b*][1,4]-7-hydrothiazine (5). To a solution of **1** (2.2 g, 0.01 mol) and AcONa (4 g, 0.048 mol) in H_2O–EtOH (300 ml, 1:1) was added 1-bromo-4-*N*-(*p*-carbethoxyphenyl)amino-2-butanone.[15] The heterogeneous mixture was stirred for 5 hr at room temperature. Tlc analysis indicated that the reaction went to completion. The beige-colored product was collected and washed with a solution of AcONa in H_2O–EtOH, H_2O, and EtOH: yield 80% (3.3 g); mp 210–214°; dec >200°; nmr 3.59 ppm; λ_{max} nm (ε × 10^3) (95% EtOH) 263 (16.6), 304 (28.7), 343 (8.5). *Anal.* ($C_{17}H_{20}N_6O_2S \cdot 0.5H_2O$) C, H, N.

2,4-Diamino-6-[2-*N*-(*p*-carboxyphenyl)amino]ethylpyrimido-[4,5-*b*][1,4]-7-hydrothiazine (6). To a solution of **1** and AcONa in

§Melting points were determined with a Gallenkamp melting point apparatus and are not corrected. Uv spectra were determined with a Cary Model 14 spectrophotometer and nmr spectra for C_7 protons were measured in DMSO-d_6 (external standard, TMS), unless otherwise indicated, with a Hitachi Perkin-Elmer Model R-20B spectrometer. Elemental analyses were carried out by Dr. H. Agahigian, Baron Consulting Co., Orange, Conn., and results obtained for elements were within ±0.4% of the theoretical values.

aqueous EtOH as described earlier was added an equimolar amount of α-bromo ketone (10) in H_2O–THF. The resultant homogeneous solution was stirred at room temperature for 2 hr and the precipitated product collected by filtration and washed with AcONa in H_2O–EtOH, H_2O, and EtOH: yield 1.1 g (65%); mp 219–221°; nmr (1 *M* Na_2CO_3 in D_2O) 3.51 ppm; λ_{max} nm (ε × 10^3) (0.01 *N* NaOH) 262 (sh, 22.5), 272 (24.0), 335 (6.1). *Anal.* ($C_{15}H_{16}N_6O_2S$) C, H, N.

4-*N*-(*p*-Carboxyphenyl)amino-2-butanone (8). A solution of methyl vinyl ketone (7 g, 0.1 mol) and *p*-aminobenzoic acid (15 g, 0.1 mol) in EtOH (200 ml) was heated under reflux for 17 hr. The mixture was filtered through charcoal when hot. Crystalline product was precipitated on standing at room temperature. After collection of a first crop by filtration, the filtrate was concentrated to give an additional crop: total yield 8.2 g (41%); mp 191–192°. *Anal.* ($C_{11}H_{13}NO_3$) C, H, N.

1-Bromo-4-*N*-(*p*-carboxyphenyl)amino-2-butanone (10). Upon addition of Br_2 (2.4 g) in AcOH (5 ml) to a heterogeneous mixture of 8 (6.0 g, 0.03 mol) in 30% HBr in AcOH (23 ml) with stirring at room temperature, the solution became homogenous. Within a few minutes a solid product began to precipitate. Stirring was continued for an additional 1 hr and Et_2O (400 ml) was added. The solid product was collected by filtration, suspended in H_2O, and stirred at room temperature for 10 min. The product was collected by filtration and washed with EtOH and Et_2O, respectively, to give 3.6 g (42%) of the desired α-bromo ketone, mp 137–139°. *Anal.* ($C_{11}H_{12}BrNO_3 \cdot 0.5H_2O$) C, H, N.

Microbiological Assay. Antibacterial activity of pyrimido[4,5-*b*][1,4]-7-hydrothiazines was determined using the *Streptococcus faecalis* system of Kisliuk.[16]

Acknowledgment. We are indebted to Mr. S. Currier for his skillful technical assistance and to Professor R. L. Kisliuk and Miss Y. Gaumont for the microbiological assay.

References

(1) S. Farber, *et al.*, *N. Engl. J. Med.*, **238**, 787 (1948).
(2) J. H. Burchenal, *Ser. Haematol.*, **1**, 47 (1965).
(3) M. C. Berenbaum, *Nature (London)*, **198**, 606 (1963).
(4) R. L. Kisliuk, *Nature (London)*, **188**, 584 (1960).
(5) S. F. Zakrzewski, M. T. Hakala, and C. A. Nichol, *Biochemistry*, **1**, 842 (1962).
(6) R. L. Blakley, "The Biochemistry of Folic Acid and Related Pteridines," North-Holland Publishing Co., Amsterdam, 1969, p 73 ff.
(7) F. L. Rose, *J. Chem. Soc.*, 3448 (1952).
(8) M. Ishidate and H. Yuki, *Chem. Pharm. Bull.*, **8**, 131 (1960).
(9) E. C. Taylor and E. E. Garcia, *J. Org. Chem.*, **29**, 2121 (1964).
(10) M. P. Nemeryuk and T. S. Safonova, *Khim. Geterosikl. Soedin.*, 470 (1966); *Chem. Abstr.*, **65**, 8903*d* (1966).
(11) H. Fenner and H. Motschall, *Tetrahedron Lett.*, 4333 (1971).
(12) T. S. Safonova and M. P. Nemeryuk, *Khim. Geterosikl. Soedin.*, 714 (1966); *Chem. Abstr.*, **67**, 64355k (1967).
(13) G. B. Elion, W. H. Lange, and G. H. Hitchings, *J. Amer. Chem. Soc.*, **78**, 2858 (1956).
(14) J. J. McCormack and H. G. Mautner, *J. Org. Chem.*, **29**, 3370 (1964).
(15) Y.-H. Kim, V. Grubliauskas, and O. M. Friedman, *J. Heterocycl. Chem.* **9**, 481 (1972).
(16) R. L. Kisliuk, *Methods Enzymol*, **18** (B), 663 (1971).

Total Synthesis and Resolution of Terreic Acid

John C. Sheehan* and Young S. Lo

Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139. *Received September 19, 1973*

Terreic acid, an antibiotic metabolite of the mold *Aspergillus terreus*, was isolated by Abraham and Florey[1a,†] in 1949. Although the antibiotic showed *in vitro* activity against gram-positive and gram-negative bacteria and fungi, *in vivo* tests were unpromising.[2] In 1958, the structure of the antibiotic was determined to be 6-epoxy-3-hydroxytoluquinone (4) by work in this laboratory.[3] The synthesis of (±)-terreic acid was reported by Rashid and Read in 1967;[4] in this note an alternative route of synthesis and the resolution of the racemic terreic acid are presented.

†The discovery that *Aspergillus terreus* produces an antibiotic substance was made by Wilkins and Harris.[1b]

Table I

	$[\alpha]^{22}D$, deg (*c*, $CHCl_3$)	Mp, °C
Natural (−)-terreic acid	−16.6 (1)[a] −26.1 (10.4)[b]	127–127.5
Synthetic (−)-terreic acid	−24.9 (1.8)	124.5–125
Synthetic (+)-terreic acid	26.5 (0.5)	124.5–125

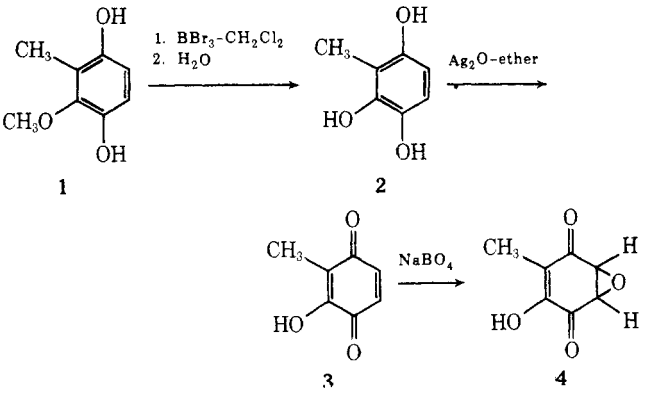
[a]Taken from ref 3. [b]Determined on a sample of natural (−)-terreic acid kindly supplied by Bristol Laboratories, Syracuse, N. Y.

Table II. Biological Activities of Terreic Acids

	Activity, %
Natural (−)-terreic acid	100
(−)-Terreic acid	90
(+)-Terreic acid	97
(±)-Terreic acid	125

The synthetic route is outlined in Scheme I. Preparation of 2-methyl-3-methoxy-1,4-hydrobenzoquinone (1) from *o*-toluidine was accomplished according to the method of Winzor.[5] The product was demethylated with boron tribromide to give a syrup (2) which was oxidized directly to 2-methyl-3-hydroxy-1,4-benzoquinone (3) by silver oxide.

Scheme I



2-Methyl-3-hydroxy-1,4-benzoquinone (3) was also the intermediate in Rashid and Read's synthesis[4] and their method was used for the formation of the epoxide 4. The spectral data for compounds 3 and 4 closely correlated with those of Rashid and Read[4] for (±)-terreic acid and those of Sheehan and coworkers[3] for naturally occurring (−)-terreic acid; however, the melting points differ somewhat.

Resolution of the (±)-terreic acid into (+) and (−) antipodes was achieved with (+)- and (−)-ephedrine,‡ respectively. The results are shown in Table I. The optical rotation of terreic acid is sensitive to solvent[3] which could explain the rotation reported in the literature. The rotations and melting points for (+)- and (−)-4 are self-consistent.

Bioassays of the racemic and resolved terreic acids gave interesting results. All samples were tested against *Staph-*

‡We thank Drs. Hiroshi Kotake, Tomoo Saito, and Kazuo Okubu who generously provided us with the *d*-ephedrine hydrochloride ($[\alpha]D$ + 34° (H_2O)) used in the resolution.