[Chem. Pharm. Bull. 28(3) 920—925 (1980)]

Stereochemical Correlation of Di- and Trihydroxy-β-diketone Fungal Metabolites, (—)-Terredionol and Terremutin Hydrate, with Sugar Alcohols: The Absolute Configuration of (—)-Terredionol[1,2]

Yoshisuke Tsuda, Tetsuji Nunozawa, Keiichi Nitta, and Yuzuru Yamamoto

Faculty of Pharmaceutical Sciences, Kanazawa University[3]

(Received October 3, 1979)

The structures, including stereochemistries, of two fungal metabolites, terremutin hydrate and (—)-terredionol, were shown to be [4*R*,5*s*,6*S*]-4,5,6-trihydroxy-2-methylcyclohexane-1,3-dione (III) and [4*R*,6*R*]-(—)-4,6-dihydroxy-2-methylcyclohexane-1,3-dione (IV) by correlating them with xylitol and [2*R*,4*R*]-(—)-pentane-1,2,4,5-tetrol, respectively.

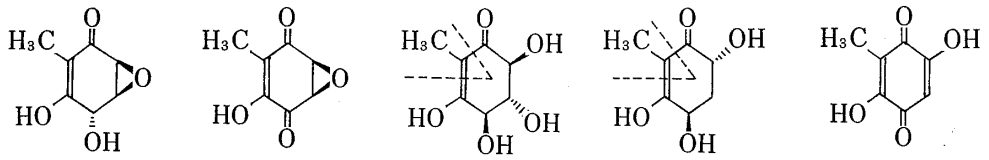
Keywords——*Aspergillus terreus*; (—)-terredionol; terremutin hydrate; fumigatin quinol hydrate; chiral cyclohexane-1,3-diones; absolute configuration; ozonolysis; [2*R*,4*R*]-(—)-pentane-1,2,4,5-tetrol; [2*R*,3*s*,4*S*]-2,3,4-trihydroxyglutaric acid

Naturally occurring cyclohexane-1,3-dione derivatives such as terremutin (I)[4] and terreic acid (II)[5] originate from polyacetates. In 1977 Yamamoto *et al.*[6] isolated two new compounds of this group, mp 189° and 152°, together with I and II, from the culture broth of *Aspergillus terreus* ATCC 12238. The former, named terremutin hydrate, was an optically inactive triol and was identified as III from its nuclear magnetic resonance (NMR) spectrum and the finding that terremutin (I) was converted to this compound on treatment with boiling water. Its optical inactivity was attributed to racemization through rapid tautomerism of the β-diketone system, suggesting the inherent meso-character of III, although strict proof of the stereochemistry of the three hydroxyl groups was lacking. The latter compound, an optically active diol ($[\alpha]_D$ −185.3° in MeOH), was suggested mainly on the basis of its NMR spectrum to be IV (the enantiomeric form is indicated in the original text[6]) or its mirror image. However, paucity of the material limited further investigations.

Biosynthetic studies of these compounds by the same authors[6] also showed that terremutin (I) was not incorporated into the diol (IV), although it was well incorporated into the triol (III), and that the biosynthetic precursor of the diol (IV) was 2,5-dihydroxy-3-methylbenzoquinone (V) (or its reductive equivalent, 2,3,5,6-tetrahydroxytoluene), which gave 20% incorporation into the diol (IV).

The present investigation was undertaken to establish rigorously the stereochemistry of these compounds by correlating them with well established carbohydrate derivatives, as well as to determine the absolute configuration of the diol, which we now designate as (—)-terredionol. This was achieved by oxidative removal of a two-carbon unit from III and IV, as indicated by dotted lines in Chart 1.

1) Part III of "Utilization of Sugars in Organic Synthesis." Part II: K. Yoshimoto, Y. Itatani, K. Shibata, and Y. Tsuda, *Chem. Pharm. Bull.*, **28**, 208 (1980).
2) This work was presented at the 99th Annual Meeting of the Pharmaceutical Society of Japan, Aug., 1979. Sapporo, Abstract 30B 2—3.
3) Location: *Takara-machi 13-1, Kanazawa, 920, Japan.*
4) M.W. Miller, *Tetrahedron*, **24**, 4839 (1968). Revised structure: G. Read and V.M. Ruiz, *J. Chem. Soc.*(*C*), **1970**, 1945.
5) J.C. Sheehan, W.B. Lawson, and R.J. Gaul, *J. Am. Chem. Soc.*, **80**, 5536 (1958).
6) N. Kiriyama, Y. Higuchi, and Y. Yamamoto, *Chem. Pharm. Bull.*, **25**, 1265 (1977).



I II III IV V

Chart 1

First, we attempted to increase the yield of (—)-terredionol from the fungus, since it was usually produced in a minute amount (5 mg/l). This problem was solved by feeding the optimum quantity of the synthetic precursor, 2,5-dihydroxy-3-methylbenzoquinone (V), to the culture medium of the fungus; the procedure increased the yield of (—)-terredionol to 50 mg/l. Excess feeding inhibited the growth of the fungus, thus decreasing the yield of(—)-terredionol.

Isolation and separation of terremutin hydrate and (—)-terredionol were carried out according to the procedure described in the previous paper,[6] as shown schematically in Chart 2, and the identities of the metabolites isolated were confirmed by direct comparison with authentic specimens.

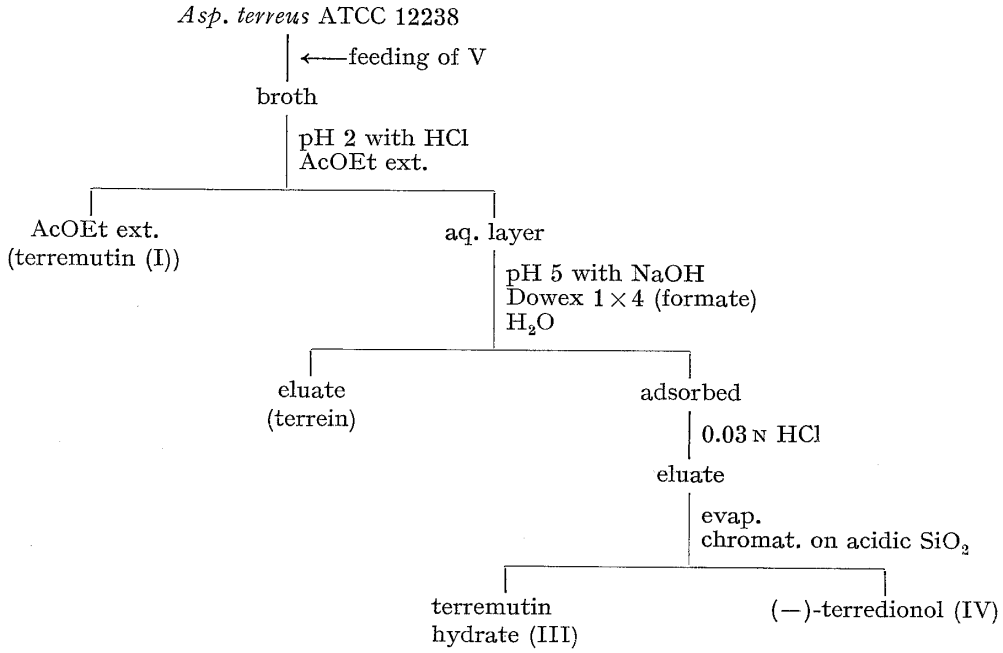


Chart 2. Isolation Procedure for the Metabolites

For removal of the two-carbon unit suggested above, we chose ozonolysis. Since terremutin hydrate (III) resisted ozonolysis in CH_2Cl_2–MeOH, it was converted to the methyl ether–triacetate (VII) by treatment with diazomethane followed by acetylation with acetic anhydride–pyridine. The methyl ether (VI), mp 129—132°, showed two separate methyl peaks in its NMR spectrum resembling a mixture of two compounds. This was, however, shown to be attributable to long-range coupling (J=2 Hz) between CH_3 and C^4-H by a decoupling technique. Irradiation at C-CH_3 changed the C^4-H signal to a clean doublet (J=7.3 Hz) and irradiation at C^4-H converted the C-CH_3 signal to a singlet. The triacetate (VII) also showed this long-range coupling (J=2 Hz). A detailed analysis of the proton signals of VI is shown in Fig. 1.

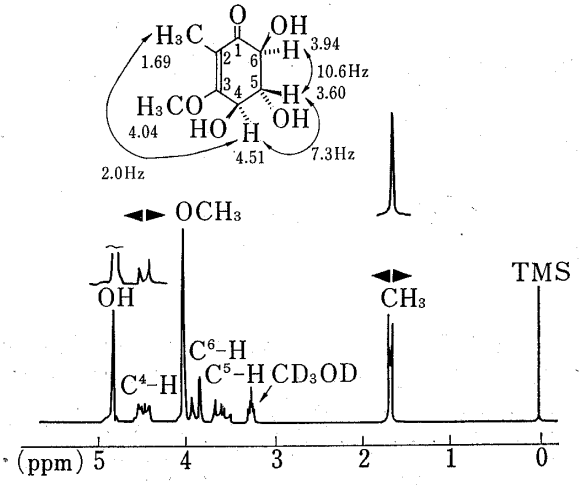


Fig. 1. 100 MHz NMR Spectrum of VI in CD_3OD

Ozonization of the triacetate (VII) in CH_2Cl_2 followed by treatment of the resulting ozonide with H_2O_2–AcOH afforded the methyl ester–acid (VIII) in satisfactory yield. Methylation of VIII with diazomethane gave a crystalline dimethyl ester (IX). The acid (VIII) and the dimethyl ester (IX) were identical with the half-ester (VIII) and (2*R*,3*s*,4*S*)-dimethyl 2,3,4-triacetoxyglutarate (IX) prepared from D-xylose *via* xylaric acid as shown in Chart 3. Conversion of IX to the pentaol (X) by lithium aluminum hydride (LAH) reduction confirmed the above assignment: the pentaol (X) and its penta-acetate (XI) were identical with xylitol and its penta-acetate, respectively, on thin layer chromatography (TLC) and gas chromatography (GC). Thus, terremutin hydrate was rigorously proved to be (4*R*,5*s*,6*S*)-2-methyl-4,5,6-trihydroxycyclohexane-1,3-dione (III).

(—)-Terredionol was similarly converted to the oily methyl ester (XIII), then to the oily diacetate (XIV). Ozonization of XIV hgave an oil, the NMR spectrum of which showed two C–CH_3 peaks of equal intensity at δ 1.63 and 1.80, suggesting that the compound was a mixture of two isomeric ozonides (XVa and XVb). Oxidation of this mixture with H_2O_2–AcOH yielded a single half-ester (XVI) as expected, which was methylated to the oily dimethyl ester (XVII), $[\alpha]_D$ −21.2° ($CHCl_3$). On LAH reduction it gave a pentanetetrol (XVIII), $[\alpha]_D$ −11.3° (water), which was characterized as the tetra-benzoate (XIX). Since (+)-pentanetetrol (3-deoxy-D-arabitol, $[\alpha]_D$ +31°[7] or +14.5°[8] in water) has already been correlated with a D-arabinoside,[7] establishing that it has (2*S*,4*S*) configuration, the absolute configuration of (—)-pentanetetrol (XVIII) should be (2*R*,4*R*). Therefore the above transformation of (—)-terredionol (IV) to (—)-pentanetetrol (XVIII) establishes the structure and the absolute configuration of (—)-terredionol as [4*R*,6*R*]-(—)-4,6-dihydroxy-2-methylcyclohexane-1,3-dione. Tautomerization of the β-diketone system does not change the absolute configuration (see Chart 5).

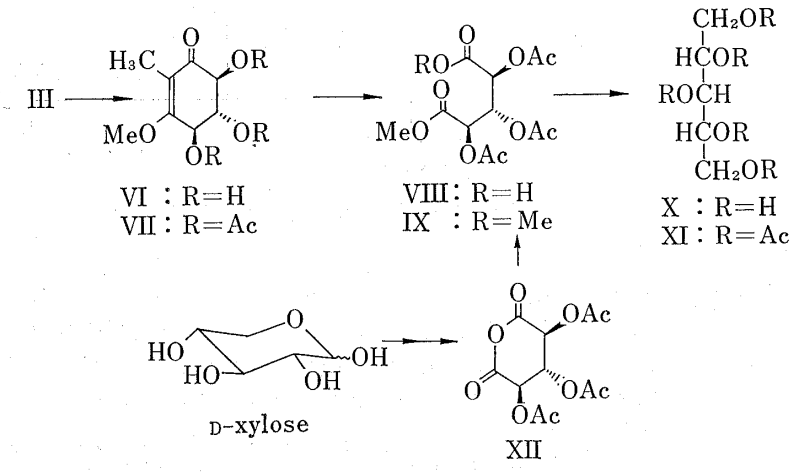
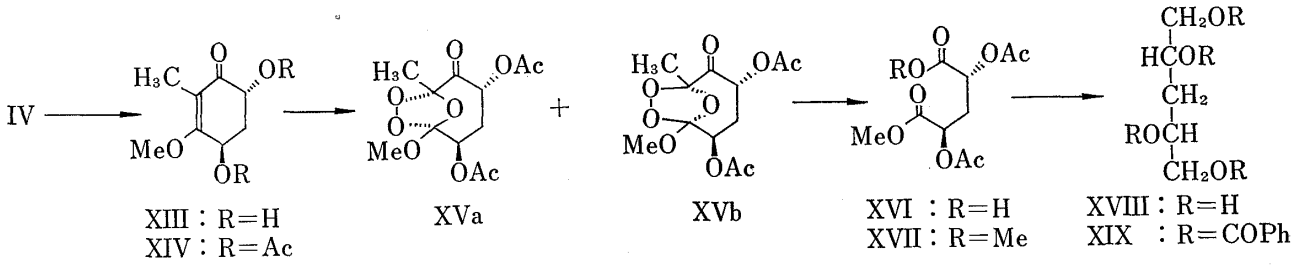


Chart 3

7) J. Davoll, B. Lithgoe, and S. Trippett, *J. Chem. Soc.*, **1951**, 2230.
8) B.M. Roth and H. Schaltegger, *Helv. Chim. Acta*, **47**, 1621 (1964).



XIII : R=H
XIV : R=Ac

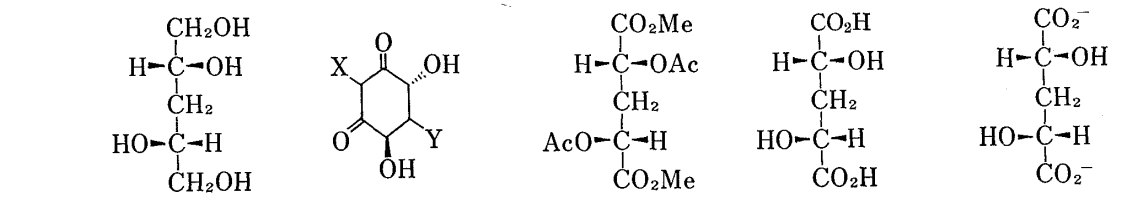
XVa

XVb

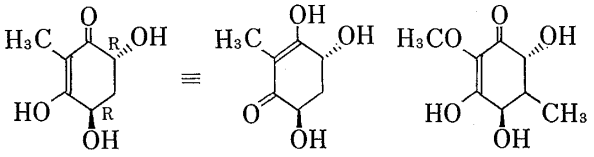
XVI : R=H
XVII : R=Me

XVIII : R=H
XIX : R=COPh

Chart 4



Configuration	[2*R*,4*R*]	[4*R*,6*R*]	[2*R*,4*R*]	[2*R*,4*R*]	[2*R*,4*R*]
Compound	1,2,4,5-pentanetetrol	4,6-dihydroxycyclohexane-1,3-dione	methyl 2,4-diacetoxyglutarate	2,4-dihydroxyglutaric acid	2,4-dihydroxyglutarate
Optical Rotation	(−)[a]	(−)[b]	(−)[b]	(−)[c]	(+)[d]



IV XX

a) J. Davoll, B. Lithgoe, and S. Trippett, *J. Chem. Soc.*, 1951, 2230. B.M. Roth and H. Schaltegger, *Helv. Chim. Acta*, 47, 1621 (1964).
b) This work.
c) J.U. Nef, *Ann.*, 376, 22 (1910).
d) L. Benoiton, M. Winitz, S.M. Birnbaum, and J.P. Greenstein, *J. Am. Chem. Soc.*, 79, 6192 (1957): Ba salt (+); J.U. Nef, *Ann.*, 376, 22 (1910): Na salt (+).

Chart 5

This finding, coupled with the results of the previous work[9] on (−)-2,4-dihydroxyglutaric acid, clarifies the stereochemical and optical relationships of these compounds as shown in Chart 5. Accordingly, fumigatin quinol hydrate (mp 166°, $[\alpha]_D$ −93° in H_2O),[10] a metabolite of *Aspergillus fumigatus* DH 413, is suggested to be [4*R*,6*R*]-(−)-4,6-dihydroxy-2-methoxy-5-methylcyclohexane-1,3-dione (XX).

Experimental

Unless otherwise stated, mp's were taken on a Yanagimoto micro hot-stage mp apparatus, UV spectra were measured with a Hitachi 323 spectrometer, IR spectra were taken as KBr discs using a Jasco IR-G spectromter, and NMR spectra were recorded on a JNM-PMX-60 (60 MHz) spectrometer with TMS as an internal reference. The following abbreviations are used: s, singlet; bs, broad singlet; bt, broad triplet; dd, doublet of doublets; t, triplet; m, multiplet. Optical rotations were measured with a Jasco DIP-SL automatic polarimeter. MS were taken on a JMS-01SG spectrometer. GC analysis was carried out with a Shimadzu GC4CM-PF gas chromatograph coupled to an FID detector, using a glass column (1 m×3 mm I.D.) packed with 1.5% OV-1 on Shimalite W (80—100 mesh), with N_2 as a carrier gas. Wakogel C-200 (silica gel) was used for column chromatography.

9) L. Benoiton, M. Winitz, S.M. Birnbaum, and J.P. Greenstein, *J. Am. Chem. Soc.*, **79**, 6192 (1957).
10) Y. Yamamoto, T. Hirai, K. Okada, and K. Saito, *Chem. Pharm. Bull.*, **22**, 83 (1974).

2,5-Dihydroxy-3-methylbenzoquinone (V)——The quinone (V) was prepared according to the procedure of Moore *et al.*[11] for the synthesis of 2,5-dihydroxy-3-ethylbenzoquinone: 30% H_2O_2 (35 ml) was added dropwise to a stirred solution of methylhydroquinone (6 g) in 60% NaOH (50 ml) at 40—50° and the stirring was continued for a further 1.5 hr. The pasty mixture was diluted with ice-water, acidified with conc. HCl, and the resulting precipitate was collected by filtration. The water layer was repeatedly extracted with Et_2O, which was pooled, dried and evaporated off to yield a solid. The solid and the above precipitate were combined and crystallized from benzene to yield V, mp 179—183° (lit.[12] mp 173—175°), as orange-red leaflets (1.7 g). IR cm^{-1}: 3300, 1620. NMR (acetone-d_6) δ: 1.90 (3H, s), 5.80 (1H, s).

Cultivation of the Fungus and Isolation of the Metabolites——*Aspergillus terreus* ATCC 12238 was grown in stationary culture at 27° in 500 ml Roux flasks containing 200 ml of the potato extract-glucose medium described previously.[6] 2,5-Dihydroxy-3-methylbenzoquinone (V; 15 mg) in 2 ml of 0.1 N Na_2CO_3 was administered to each flask on the 7th day of cultivation. After further cultivation for 5 days, the mycelia were filtered off and the broth (6 liters) was adjusted to pH 2 with HCl then washed 4 times with AcOEt. The water layer was brought to pH 5 with NaOH, concentrated to *ca.* 2 liters under reduced pressure at 40°, and passed through a column of Dowex 1×8 (formate, 4×44 cm). The column was washed with H_2O (4 l) and then eluted with 0.03 N HCl. The effluent having λ_{max} 265 nm was collected and evaporated to dryness under reduced pressure at 40°. The residue was chromatographed on acidic silica gel (Mallinckrodt, Silic AR CC-4), eluting with $CHCl_3$–MeOH (20: 1). Crystallizations of the first several fractions from AcOEt gave (−)-terredionol (IV) as colorless needles, mp 162—165°[13] (140 mg). IR cm^{-1}: 3500, 3230, 3120, 1585. NMR (THF-d_8) δ: 1.64 (3H, s), 2.04 (2H, t, J=6 Hz), 4.24 (2H, t, J=6 Hz).

The following several fractions afforded a mixture of III and IV, from which further crops of IV (160 mg) and III (300 mg) were isolated by repeated chromatography.

Further elution with the same solvent gave terremutin hydrate (III) as colorless prisms on crystallization from MeOH–$CHCl_3$, mp 199—201°[13] (700 mg). IR cm^{-1}: 3400, 3250, 1615. NMR (D_2O, internal reference, $Me_3SiCD_2CD_2COONa$) δ: 1.70 (3H, s), 3.63 (1H, dd, J=8 and 11 Hz), 4.23 (2H, dd, J=8 and 11 Hz).

The Methyl Ether (VI)——Terremutin hydrate (III, 200 mg) in MeOH (3 ml) was treated with excess ethereal diazomethane for 4 hr with stirring. Removal of the solvent and crystallization of the residue from MeOH–Et_2O gave the methyl ether (VI), mp 129—130° (lit.[6] mp 128—129°), as colorless needles (89 mg). IR cm^{-1}: 3280, 1648, 1610. NMR: see Fig. 1.

The Methyl Ether-triacetate (VII)——A mixture of the methyl ether (VI; 80 mg) and Ac_2O (1 ml) in pyridine (2 ml) was kept overnight at room temp. The mixture was then poured into water and extracted with Et_2O. The Et_2O fraction was washed with water, dried over Na_2SO_4, and concentrated. Chromatography of the gummy residue in $CHCl_3$ and crystallization of the eluate from *n*-hexane–Et_2O gave the triacetate (VII), mp 70—74°, as colorless prisms (61 mg). IR cm^{-1}: 1758, 1677, 1618. NMR ($CDCl_3$) δ: 1.80 (3H, d, J=2 Hz), 2.05 (3H, s), 2.10 (3H, s), 2.13 (3H, s), 3.76 (3H, s), 5.23—5.65 (2H), 5.96 (1H, m).

Ozonolysis of the Methyl Ether-triacetate (VII)——Oxygen containing ozone was passed through a cooled solution of VII (150 mg) in CH_2Cl_2 (15 ml) for 5 hr and the solution was kept overnight at 0°, then concentrated to dryness *in vacuo*. The residue in AcOH (3 ml) was treated with 30% H_2O_2 (0.4 ml) under stirring for 9 hr at room temp., then concentrated to dryness *in vacuo*. The residue was taken up in Et_2O and extracted with 5% $NaHCO_3$. Acidification of the basic extract with 5% HCl and extraction with AcOEt gave, on removal of the solvent from the dried extract, a monomethyl ester (VIII) as an oil (100 mg). NMR ($CDCl_3$) δ: 2.10 (3H, s), 2.15 (6H, s), 3.74 (3H, s), 5.35 (2H, d, J=4.5 Hz), 5.65 (1H, diffused t, J=4.5 Hz), 8.23 (1H, bs).

The Dimethyl Ester (IX)——The above monomethyl ester (VIII, 100 mg) was treated with an excess of ethereal diazomethane for 1 hr to give the dimethyl ester (IX), which, on purification by preparative TLC, crystallized in prisms, mp 74—77°. GC: t_R 8.3 min (temp., 130°; flow rate, 60 ml/min). IR cm^{-1}: 1750. NMR ($CDCl_3$) δ: 2.08 (3H, s), 2.15 (6H, s), 3.73 (6H, s), 5.30 (2H, d, J=4.5 Hz), 5.67 (1H, diffused t, J=4.5 Hz). This was identical with (2*R*,3*s*,4*S*)-dimethyl 2,3,4-triacetoxyglutarate (see below) on the basis of comparisons of mp, TLC, IR, NMR, and GC.

Xylitol (X) from the Dimethyl Ester (IX)——The dimethyl ester (IX; 35 mg) and $LiAlH_4$ (80 mg) in tetrahydrofuran (6 ml) were heated under reflux for 6 hr. The mixture was decomposed with 5% HCl and poured onto a column of Amberlite IR-120 (H^+-form). Elution of the column with H_2O and concentration of the eluate *in vacuo* gave a gummy residue identical with xylitol (X) on TLC. This was acetylated with Ac_2O and pyridine to give the pentaacetate (XI) which had t_R=7.8 min on GC (temp. 150°; flow rate, 50 ml/min). Authentic samples for comparison showed the following t_R's: xylitol pentaacetate 7.8 min, D-arabitol pentaacetate 7.2 min, adonitol pentaacetate 7.2 min.

11) R.E. Moore, H. Singh, and P.J. Scheuer, *J. Org. Chem.*, **31**, 3645 (1966).

12) W.G. Hanger, W.C. Howell, and A.W. Johnson, *J. Chem. Soc.*, **1958**, 496

13) Authentic samples showed the following mp's under the same conditions: (−)-terredionol, mp 162—164°; terremutin hydrate, mp 197—199°. In an open capillary they gave mp's of 152° and 189°, respectively.[6]

(2*R*,3*s*,4*S*)-2,3,4-Triacetoxyglutaric Anhydride (XII)[14]——D-Xylose (10 g) was oxidized with conc. HNO_3 (17 ml) and $NaNO_2$ (20 mg) for 2 hr at 55—65° and the product was converted to the Zn salt (6.6 g), then to (2*R*,3*s*,4*S*)-2,3,4-triacetoxyglutaric anhydride (XII), mp 142—146° (lit.[14] mp 146—147°). IR cm^{-1}: 1830.

(2*R*,3*s*,4*S*)Dimethyl 2,3,4-Triacetoxyglutarate (IX)——The anhydride (XII) in MeOH was heated at 60° for 1 hr, then concentrated to dryness. The residue was taken up in Et_2O, and extracted with 5% $NaHCO_3$. On acidification with 5% HCl and extraction with Et_2O, this gave the monomethyl ester (VIII). On crystallization from *n*-hexane–ether it showed mp 122—125°. The NMR spectrum was superimposable on that of the half-ester (VIII) obtained by ozonolysis of VII. Treatment of VIII with excess ethereal diazomethane afforded the dimethyl ester, mp 77—78° (lit.[15] oil), which was identical with the compound described above. *Anal.* Calcd for $C_{13}H_{17}O_{10}$: C, 46.71; H, 5.43. Found: C, 46.35; H, 5.32.

The Methyl Ether-diacetate (XIV)——(−)-Terredionol (IV; 160 mg) in MeOH (1 ml) was treated with excess ethereal diazomethane for 2 hr. Removal of the solvent left the methyl ether (XIII) as an oil. NMR ($CDCl_3$) δ: 1.73 (3H, s), 1.85—2.75 (2H), 4.30 (3H, s), 4.46 (1H, dd, J=6 and 12 Hz), 4.80 (1H, bs). This was acetylated with Ac_2O (1.5 ml) and pyridine (3 ml) overnight at room temp. Work-up as usual and chromatography of the product in $CHCl_3$ yielded the diacetate (XIV) as an oil (86 mg). $[\alpha]_D^{15}$ −119.5° (c=0.95 in $CHCl_3$). UV λ_{max}^{EtOH}: 265 nm. IR ($CHCl_3$) cm^{-1}: 1740, 1670, 1630. NMR ($CDCl_3$) δ: 1.75 (3H, s), 2.16 (6H, s), 2.33 (2H, dd, J=3 and 7 Hz), 3.76 (3H, s), 5.52 (1H, dd, J=7 and 10.5 Hz), 5.90 (1H, bt, J=3 Hz).

Ozonolysis of the Methyl Ether-diacetate (XIV)——The methyl ether-diacetate (XIV; 80 mg) in CH_2Cl_2 (20 ml) was ozonized as described above for 3 hr at 0°. Removal of the solvent *in vacuo* left an oily ozonide (XVa and XVb). NMR ($CDCl_3$) δ: 1.63 and 1.80 (3H, s and s), 2.13 (6H, s), 4.06 (3H, s). This was treated with AcOH (3 ml) and 30% H_2O_2 (0.4 ml) for 4.5 hr at room temp. then concentrated to dryness. Work-up as above gave the monomethyl ester (XVI; 85 mg) as an oil. NMR ($CDCl_3$) δ: 2.15 (6H, s), 2.43 (2H, dd, J=6.5 and 7 Hz), 3.73 (3H, s), 5.13 (2H, t, J=7 Hz), 9.20 (1H, bs).

The Dimethyl Ester (XVII)——The above monomethyl ester (XVI; 85 mg) was treated with an excess of ethereal diazomethane for 2 hr and the product was purified by chromatography in CH_2Cl_2 to give the dimethyl ester (XVII) as an oil. $[\alpha]_D^{18}$ −21.2° (c=3.07 in $CHCl_3$). IR ($CHCl_3$) cm^{-1}: 1750. NMR ($CDCl_3$) δ: 2.14 (6H, s), 2.36 (2H, dd, J=6 and 8 Hz), 3.70 (6H, s), 5.10 (2H, dd, J=6 and 8 Hz).

(−)-Pentanetetrol (XVIII)——The dimethyl ester (XVII; 30 mg) and $LiAlH_4$ (100 mg) in THF (4 ml) were heated at 80° for 6 hr. The mixture was decomposed by the addition of a few drops of water, filtered, and the residue extracted thoroughly with a 2:1 mixture of THF and water, then with 50% EtOH. Concentration of the combined filtrate and extracts left a solid, which was extracted with hot EtOH to give the tetrol (XVIII) as a solid. $[\alpha]_D^{18}$ −11.3° (c=1.6 in H_2O). This was dissolved in pyridine (4 ml) and treated with excess benzoyl chloride at 50° for 20 hr. The mixture was poured into water and extracted with Et_2O. The Et_2O extract was washed with 0.5 N HCl, 5% $NaHCO_3$, and water, then dried over Na_2SO_4 and concentrated to dryness. Chromatography of the residue gave the tetrabenzoate (XIX) as a gum. MS *m/e*: 430 ($M^+ - C_6H_5COOH$). NMR ($CDCl_3$, 100 MHz) δ: 2.38 (2H, dd, J=6 and 7 Hz), 4.60 (4H, $OCH_2 \times 2$), 5.69 (2H, m, $CH \times 2$), 7.2—8.0 (20H, $Ph \times 4$).

14) M.L. Wolfrom and E. Usdin, *J. Am. Chem. Soc.*, **75**, 4318 (1953).
15) E. Hardegger, K. Kreis, and D. Spitz, *Helv. Chim. Acta*, **35**, 958 (1952).