With pyrogallol and maleic anhydride 1,2-bis(2′,3′,4′-trihydroxybenzoyl)ethylene (Vb) was obtained. Phenol, hydroquinone, orcinol, and phloroglucinol did not react.

Experimental

All melting points are uncorrected and were determined in an electrically heated copper block.

Preparation of Hydroxy Aromatic Ketones Using Cation-Exchange Resin.—Equimolecular quantities of the phenol and acid anhydride or carboxylic acid were heated together with that weight of resin (preparation given in part I[3], except that drying lasted for 6 hr.) which is about 45% by weight of the phenol in an oil bath at 160° with constant stirring for 2–3 hr. The solution was then allowed to cool and ethanol was added to extract the reaction product. The filtrate was refluxed with a few drops of concentrated hydrochloric acid reduced to a low volume *in vacuo* and was washed with water to remove any unreacted phenol. When aromatic acids or their anhydrides were used the residue obtained, after removal of the alcohol, was treated with sodium bicarbonate. When resorcinol and isobutyric acid were allowed to react as above an oil separated, and this, on vacuum distillation, gave solid resoisobutyrophenone.

Preparation of Hydroxy Aromatic Ketones Using 1 Drop of Concentrated Sulfuric Acid.—Since the method was published in a journal[2] not readily accessible, a brief description is given here. Equimolecular quantities of the phenol and the acid anhydride were refluxed with 1 drop of concentrated sulfuric acid or polyphosphoric acid for about 10–15 min. (in the case of orcinol and butyric anhydride, 30 min.). The reaction product was poured into water, a small quantity of ethanol and a few drops of concentrated hydrochloric acid were added, and the solution was refluxed for 20–30 min. After removal of the water and alcohol by distillation the solid hydroxy ketone separated out.

The following hydroxy ketones were obtained (melting point, yield): gallobutyrophenone (98–100°, 64.5%); α-orcibutyrophenone (120–122°, 16%); phlorobutyrophenone (184–186°, 32%); 5-chlororestacetophenone (176–177°,[6] 12%); and gallobenzophenone (140–142°, 14%).

Resorcinol Diacetate and Its Conversion to Resacetophenone.—Four grams of resorcinol and either 4 g. of acetic anhydride or 6 g. of acetic acid were heated at about 100° for 15 min. with 2 g. of resin when the solution became orange in color. The product was extracted with ethanol and filtered off from the resin. The ethanol and unreacted anhydride or acid were distilled off and a liquid was obtained, b.p. 164° (5 mm.), which proved to be resorcinol diacetate. Five grams of the latter were heated with 3 g. of resin at 160° for 1 hr. when a dark red product was obtained. After extraction with ethanol it was filtered off from the resin and on suitable treatment yielded 2.5 g. (64%) resacetophenone.

Preparation of Resacetophenone Using Acetyl Chloride.—Resorcinol (5.6 g.), 4.5 g. of acetyl chloride, and 2 g. of cation-exchange resin were refluxed for 2 hr. The reaction product after suitable treatment yielded 4.0 g. (52%) of resacetophenone.

Preparation of 1,2-Bis(hydroxybenzoyl)ethylenes.—Four grams of resorcinol and 4 g. of maleic anhydride were dissolved in 50 g. of ethylene dichloride, 5 g. of cation exchange resin was added, and the mixture was refluxed for 2 hr. when a solid separated. The ethylene dichloride was filtered off, ethanol was added to dissolve the solid, and the resin was removed by filtration. After the removal of the ethanol, the residue was shown to consist of a mixture of 0.5 g. of fumaric acid and 2.1 g. (19%) of 1,2-bis(2′,4′-dihydroxybenzoyl)ethylene, m.p. 254–256° (dec.), identical with that obtained by Rao, *et al.*[7] In a similar way, 4 g. of pyrogallol gave 2.0 g. of 1,2-bis(2′,3′,4′- trihydroxybenzoyl)-ethylene, m.p. 236–238°, identical with that obtained by Bogert and Ritter.[8]

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Phenolic Metabolite of "Low-Iron Fermentation" of *Streptomyces griseus*. Characterization of 2,3-Dihydroxybenzoic Acid

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In studies[1,2] dealing with the relationship of streptomycin biogenesis to some oxidation–reduction systems operative in *Streptomyces griseus*, strain Z-38, it was observed that, with a substantial elimination of cytochromes due to reduced iron content in the fermentation medium, a distinct inhibition of streptomycin formation resulted and a phenolic metabolite of unknown structure accumulated. The compound was isolated as an amorphous solid and was considered to be a derivative of an intermediate in the biosynthesis of streptomycin since it suppressed, after its addition to an iron(II)-containing medium, the production of streptomycin. This effect could be reversed by the addition of streptidine.

The crude yellow amorphous sodium salt[3] of the phenol was converted to the free phenol, which was purified by treatment with carbon and was crystallized from hot water. The colorless compound was also purified by sublimation, m.p. 204–205° (cor.). Analytical data indicated the empirical formula $C_7H_6O_4$ for the phenol and the similarity of the melting point with that reported for 2,3-dihydroxybenzoic acid (205°) suggested the identity of the phenol. This suggestion was confirmed by the fact that the unknown phenol and 2,3-dihydroxybenzoic acid[4] had superimposable n.m.r.[5,6] and infrared spectra.

The metabolite, 2,3-dihydroxybenzoic acid, has been isolated as its glycine conjugate from low-iron fermen-

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(4) We thank Dr. J. B. Neilands of the University of California for this sample.

(5) We are grateful to Mr. W. S. Fleming for determining the n.m.r. spectra.

(6) The identity of the unknown phenol was suggested before analytical data were obtained by analysis of its n.m.r. spectrum. First-order[7] analysis of the spectrum of the unknown phenol (2,3-dihydroxybenzoic acid) gave the following absorption positions and coupling constants for the protons present: H-4, τ 3.00; H-5, τ 3.20; H-6, τ 2.74; $J_{4,5}$ = 8.0 c.p.s.[8]; $J_{4,6}$ = 2.6 c.p.s.; $J_{5,6}$ = 7.0 c.p.s. As an aid in the interpretation of the n.m.r spectrum of the unkown phenol, the spectra of several model compounds were analyzed. First-order analysis of the spectrum of 2,4-dihydroxybenzoic acid gave H-3, τ 3.75; H-5, τ 3.66; H-6, τ 2.37; $J_{3,5}$ = 2.3 c.p.s.; $J_{3,6}$ = 0.6 c.p.s.; $J_{5,6}$ = 8.2 c.p.s. First-order analysis of the spectrum of 2,5-dihydroxybenzoic acid gave H-3, τ 3.31; H-4, τ 3.10; H-6, τ 2.77; $J_{3,4}$ = 8.9 c.p.s.; $J_{3,6}$ = 0.8 c.p.s.; $J_{4,6}$ = 2.7 c.p.s. Analysis of these spectra was greatly simplified by the fact that H-6 in all three compounds is strongly deshielded by the carboxylate anion. Hence the absorptions due to these protons occur at lower field than, and are well separated from, the other absorptions present.

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tation broths of *Bacillus subtilis*,[9] from cultures of *Aspergillus niger*,[10] and as a product of tryptophane metabolism by *claviceps Paspali*.[11] In general the *o*-dihydric phenolic structure is rare among microbial products.

Experimental

A sample (40 mg.) of the crude yellow amorphous phenolic metabolite was dissolved in a minimum quantity of boiling water. The aqueous solution was acidified with sulfuric acid and the free phenol was extracted with ethyl acetate. The ethyl acetate solution was treated with Darco G-60 activated carbon, filtered, and evaporated. The product was recrystallized twice from hot water. When the amorphous or colorless crystalline phenol was heated on a Kofler hot stage, it began to sublime at 140° and formed beautiful hard cubes, m.p. 204–205° (cor.). A mixture melting point determination with an authentic sample of 2,3-dihydroxybenzoic acid produced no melting point depression.

Anal. Calcd. for $C_7H_6O_4$: C, 54.55; H, 3.92; O, 41.53. Found: C, 54.77; H, 3.94; O, 41.29.

Nuclear magnetic resonance spectra were determined using a Varian A-60 instrument; tetramethylsilane served as an external reference. Solutions were prepared using 10 mg. of the sample and 0.2 ml. of deuterium oxide; sufficient anhydrous potassium carbonate was added so that the sample dissolved. The final solutions were deoxygenated before the spectra were determined.

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Essential Oils and Their Constituents. XXI.[1] Isomerization of α-Humulene Monoxide to β-Humulen-7-ol. A New Sesquiterpene Alcohol

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During a recent study of the volatile components of wild ginger (*Zingiber zerumbet* Smith), the authors observed formation of a sesquiterpene alcohol when the essential oil or *l*-humulene monoxide, one of its constituents, was chromatographed over active alumina.[3] It is the purpose of this Note to present experimental evidence which established its structure.

The infrared spectrum of the compound, $C_{15}H_{24}O$ (shown in Fig. 1), displayed absorptions characteristic of a free hydroxyl group (3390 cm.$^{-1}$), an exocyclic methylene group >C=CH$_2$ (895 and 1642 cm.$^{-1}$), a trisubstituted double bond >C=CH— (835 cm.$^{-1}$), and a *trans*-symmetrical disubstituted double bond —CH=CH— (975 cm.$^{-1}$). In accordance with these structural features of the molecule, 3 moles of hydrogen were consumed during hydrogenation with Adams catalyst. The ultraviolet absorption spectrum of the compound was practically featureless, suggesting absence of conjugated unsaturation.

Column chromatography of the hydrogenated product over neutral grade I alumina followed by gas chromatography of petroleum ether eluates revealed the presence of humulane (II, Fig. 2) as a product of hydrogenolysis, the identity of the hydrocarbon being established by comparison of its retention time on both Reoplex 400 and SAIB, respectively, as well as by comparison of its infrared absorption spectrum with that of an authenticated sample prepared *via* hydrogenation of pure α-humulene (V). Similar examination of diethyl ether eluates showed the presence of two isomeric saturated alcohols (III), emerging after 19.8 and 23.4 min., respectively, from the Reoplex column operated at 200°.

Chromic acid oxidation converted the alcohols to the corresponding saturated ketones (VI) isomeric with regard to the spatial arrangement of the methyl groups at C-4 and C-8 positions. These ketones were resolved by preparative gas chromatography. Their retention times, indicative of the corresponding boiling points of the two compounds, and refractive indices would permit the following tentative configurational assignments: *cis*—retention time of 32.6 min., $n^{25}D$ 1.4735; and *trans*—retention time of 30.4 min., $n^{25}D$ 1.4729. Of the two ketones only the *cis* isomer yielded a crystalline semicarbazone (m.p. 190–191°).

Formation of the alcohol during column chromatography of humulene monoxide (IV)[3] suggested an allylic humulane-type skeleton of the molecule. The assumption was confirmed by examination of the reaction product obtained on refluxing the epoxide with pyridinium bromide. Caryophyllene oxide so treated has been reported to yield a mixture of allylic alcohols.[4] Gas chromatographic analysis of the humulene monoxide–pyridinium bromide isomerization product showed that its major component displayed the same retention time as the alcohol obtained *via* either alumina-catalyzed isomerization of α-humulene 7,8-epoxide or column chromatography of oil of wild ginger. The infrared absorption spectra of the three alcohols proved to be identical as well. The course of the chemical reactions is illustrated in Fig. 2 and relevant experimental data are recorded in Table I.

TABLE I
CRITERIA OF IDENTITY FOR SESQUITERPENE ALCOHOL OBTAINED BY THREE DIFFERENT ROUTES

Method of preparation	$n^{25}D$	$[\alpha]^{25}D$	Retention time,[a] min.
(1) Column chromatography of oil of wild ginger	1.5135	+25.81°	29.5
(2) Isomerization of *dl*-humulene monoxide over alumina	1.5135	±0.0	29.5
(3) Isomerization of *dl*-humulene monoxide with pyridinium bromide	1.5140	±0.0	29.5

[a] Column, Reoplex 400; temperature 200°.

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