IR ($CHCl_3$) 3575 $cm^{-1}$ (OH); NMR ($CDCl_3$) $\delta$ 0.90, 1.16, 1.41 (s, 9, $Me_3$), 3.50 (d, 1, $J$ = 3Hz, H-3), 3.58, 4.62 (dd, 2, $J$ = 11 Hz, H-19), 6.49 (s, 1, benzyl H), 7.2–7.7 (m, 5 H, aromatic Hs).

Anal. Calcd for $C_{27}H_{36}O_3$: C, 79.37; H, 8.88. Found: C, 79.50; H, 8.58.

**2-Epivirescenol A (8a).** A mixture of 0.25 g of **3c** and 5 mL of 0.1 N methanolic sulfuric acid in 5 mL of chloroform was refluxed for 5 h. It was diluted with water and extracted with chloroform. The extract was washed with water, dried, and concentrated under vacuum. Chromatography of the residue (90 mg) on silica and elution with 6:1 benzene/ethyl acetate gave 0.13 g (65%) of a solid whose crystallization from 1:1 benzene/petroleum ether yielded crystalline **8a:** mp 168–170 °C; IR ($CHCl_3$) 3570 $cm^{-1}$ (OH); NMR ($CDCl_3$) $\delta$ 0.85, 1.05, 1.20 (s, 9, $Me_3$), 3.38 (m, 1, H-3), 3.48, 4.63 (dd, 2, $J$ = 11 Hz, H-19).

Anal. Calcd for $C_{20}H_{32}O_3$: C, 74.96; H, 10.06. Found: C, 74.62; H, 10.30.

A solution of **8a** (0.20 g) in $Me_2SO$ (5 mL) was stirred under nitrogen at 160 °C for 4 h. It then was poured into water and the mixture was extracted with chloroform. The extract was washed with water, dried, and concentrated under vacuum. Chromatography of the residue (0.16 g) on silica and elution with 6:1 benzene/ethyl acetate gave 0.14 g of **2a** (vide supra).

A solution of virescenol A (**1a**) (0.20 g) was treated in the same manner. The same workup gave 50 mg of **2a** (vide supra).

**Keto Ether 2b.** A solution of hydroxy ether **2a** (0.2 g) in methylene chloride (5 mL) was added dropwise (10–15 min) to a suspension of Collins reagent (0.7 g) in anhydrous methylene chloride (10 mL). The mixture was allowed to stir for an additional 15 min and filtered. The dark filtrate was washed successively with 1 N sulfuric acid and water and concentrated under vacuum. The light brown residue was purified by chromatography on silica. Elution with chloroform led to 0.2 g (100%) of oily **2b:** IR ($CCl_4$) 1762 $cm^{-1}$ (C=O); NMR ($CDCl_3$) $\delta$ 0.91, 1.00, 1.30 (s, 9, $Me_3$), 3.78, 4.39 (dd, 2, $J$ = 11 Hz, H-19), 4.04 (d, 1, $J$ = 6 Hz, H-2).

Anal. Calcd for $C_{20}H_{28}O_2$: C, 79.95; H, 9.39. Found: C, 80.15; H, 9.17.

**Reduction of Keto Ether 2b.** To a solution of **2b** (0.40 g) in methanol (20 mL) was added a solution of sodium borohydride (90 mg) in 6 mL of 50% aqueous methanol. The mixture was stirred at room temperature for 10 min and then 50 mL of 0.5 N sulfuric acid solution was added thereto. It was extracted with chloroform and the extract was washed with saturated sodium bicarbonate solution, dried, and concentrated under vacuum. Chromatography of the residue on silica and elution with 50:1 chloroform/methanol gave 0.38 g (94%) of oily **2c:** IR ($CCl_4$) 3650 $cm^{-1}$ (OH); NMR ($CDCl_3$) $\delta$ 0.83, 0.88, 1.04 (s, 9, $Me_3$), 3.36, 3.96 (dd, 2, $J$ = 9.5 Hz, H-19), 3.76 (d, 1, $J$ = 6 Hz, H-3), 4.10 (m, 1, H-2).

Anal. Calcd for $C_{20}H_{30}O_2$: C, 79.42; H, 10.00. Found: C, 79.35; H, 9.85.

A solution of 0.16 g of ketone **2b** in 10 mL of 9:1 tetrahydrofuran/ethanol was added over a 10-min period to a solution of lithium (15 mg) in 30 mL of liquid ammonia and the reaction mixture was stirred at −40 °C for 10 min. A few drops of bromobenzene were added to the mixture, the ammonia was evaporated in a stream of nitrogen, and 20 mL of a 0.5 N sulfuric acid solution was added to the residue. The resulting mixture was extracted with chloroform and the extract combined, washed with water, dried, and concentrated under vacuum. Chromatography of the residue (0.12 g) on silica and elution with 9:1 benzene/ethyl acetate gave 25 mg of **2c** (vide supra). Elution with 6:1 benzene/ethyl acetate gave 35 mg of **2a** (vide supra) and 30 mg of virescenol B (**1b**), identical in all respects with an authentic sample.[1]

**Tosylation of Virescenol A (1a).** A solution of 0.96 g of **1a** and 0.70 g of tosyl chloride in 10 mL of pyridine was stirred at room temperature for 72 h. It was poured into ice water and extracted with chloroform. The extract was washed with 1 N hydrochloric acid and water, dried, and concentrated. Chromatography of the residue (1.2 g) on silica and elution with 9:1 benzene/ethyl acetate gave 0.25 g (13%) of virescenol A 2,19-ditosylate (**1d**) [IR ($CHCl_3$) 3540 $cm^{-1}$ (OH); NMR ($CDCl_3$) $\delta$ 0.86 (s, 6, $Me_2$), 1.03 (s, 3, Me), 2.43 (s, 6, aromatic $Me_2$), 3.26 (d, 1, $J$ = 10 Hz, H-3), 3.96 (s, 2, H-19), 4.73 (m, 1, H-2), 7.1–7.8 (m, 8, aromatic Hs)], 0.18 g (19%) of tetrahydrofuran **2a** (vide supra), and 0.40 g (28%) of virescenol A 19-tosylate (**1c**) [IR ($CHCl_3$) 3575 $cm^{-1}$ (OH); NMR ($CCl_4$) $\delta$ 0.84 (s, 6, $Me_2$), 1.50 (s, 3, Me), 2.38 (s, 3, aromatic Me), 7.3–7.6 (dd, 4 H, $J$ = 8 Hz, aromatic Hs)].

**Registry No.**—**1a,** 22343-46-9; **1b,** 22343-47-1; **1c,** 63089-00-9; **1d,** 63089-01-0; **1e,** 63089-02-1; **2a,** 63089-03-2; **2b,** 63089-04-3; **2c,** 63121-82-4; **3a,** 63089-05-4; **3b,** 63089-06-5; **5a,** 63089-07-6; **5b,** 63089-08-7; **6a,** 63089-09-8; **6b,** 63089-10-1; **8a,** 63089-11-2; benzaldehyde, 100-52-7.

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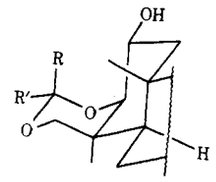
(2) The $\Delta^{8(9)}$ isomer of **2a** has been shown previously to accompany the tosylates $\Delta^{8(9)}$-**1c** and $\Delta^{8(9)}$-**1d** as a product of the derivatization of isovirescenol A ($\Delta^{8(9)}$-**1a**) [J. Polonsky, Z. Baskevitch, N. Cagnoli Bellavita, and P. Ceccherelli, *Bull. Soc. Chim. Fr.*, 1912 (1970)].

(3) This substance probably possesses the benzylic $\beta$-hydrogen configuration, depicted in conformational structure i.




i, R = H; R′ = $C_6H_5$
ii, R = R′ = Me

(4) This ketal can be represented by structure ii, in which an energetically unfavorable, 1,3-diaxial, nonbonded interaction exists between the ketal $\beta$-methyl group and the C(2)–C(3) bond. This may be responsible for the production of two ketals, but only one acetal.

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# One-Vessel Synthesis of 4-Hydroxyproline from Glyoxal and Oxaloacetic Acid[1]


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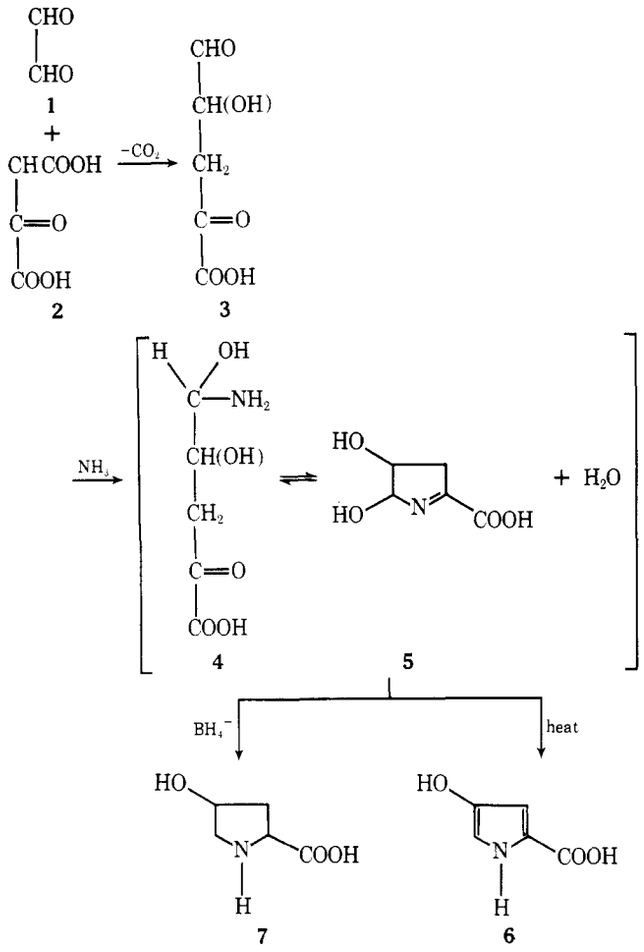
4-Hydroxyproline, obtained initially by isolation from gelatin hydrolyzates,[2] was first synthesized by Leuchs,[3] and subsequently by a variety of other procedures. Some of these were variants of the Leuchs method involving a valerolactone intermediate;[4–8] others were based on different routes,[9–12] but generally required rather complex reaction sequences, commercially unavailable starting materials, or necessitated the isolation of intermediates.

We report here a new synthesis of the mixed racemates of 4-hydroxyproline, obtained in good yield from the commercially available starting compounds, glyoxal and oxaloacetic acid. Although this procedure was briefly cited earlier[13] in connection with the use of one of the intermediates (**3,** Scheme I), no details were presented. The entire reaction sequence is carried out in a single vessel, the yield is approximately 40% (mixture of racemates), and the starting materials have the advantage that at least one can be obtained readily in radioactive form,[14] since the goal in synthesizing 4-hydroxyproline is usually to obtain the radioactive (especially, the $^{14}C$) product.

## Results and Discussion

In outline, this procedure involves simply the reaction of equimolar glyoxal and oxaloacetic acid in aqueous solution at room temperature and neutral pH, and the subsequent addition, successively, of excess $NH_4OH$ and sodium borohydride. Our speculation concerning intermediate steps is outlined in Scheme I, as supported by previous reports of analogous synthetic steps, involving the condensation of glyoxylic acid and oxaloacetic acid to form 4-hydroxy-2-ketoglutaric acid[15,16] (in analogy with **3,** Scheme I), the formation of pyrrole-2-carboxylic acid[17] (in analogy with **6,** Scheme I),

Scheme I




or the conversion of 2-ketoglutaric semialdehyde to proline[17,18] (in analogy with the conversion of **3** to **7**, Scheme I).

The rather involved series of column purification steps described below were aimed at establishing the products as crystalline *trans*- and *cis*-4-hydroxyproline. For the isolation of radioactive products on a small scale, it would seem sufficient simply to carry out the first desalting step (Dowex 50 $H^+$ chromatography) followed by separation of the two diastereomeric forms on an amino acid analyzer type column.

## Experimental Section

Starting materials were glyoxal trimeric dihydrate and oxaloacetic acid (both from Sigma Chemical Co.). Sodium borohydride was a British Drug House product, 4-hydroxy-L-proline and allo-4-hydroxy-D-proline were purchased from Sigma Chemical Co. [2-$^{14}$C]-4-Hydroxy-DL-proline ($4 \times 10^7$ dpm/μmol) was purchased from Amersham Searle Corp. Resins used were Dowex 50-W X-8, 100–200 mesh (Sigma); AG-1-X8, 100–200 mesh, and AG-50W X12, 400 mesh, both from BioRad Laboratories.

Paper electrophoresis was carried out at pH 1.85 at 260 V/cm.[19] Amino acid analysis was carried out by the methods and with the buffer system described earlier;[20] by this method, *trans*-4-hydroxyproline and *cis*-4-hydroxyproline are eluted at approximately 65–70 and 80–85 min, respectively. Colorimetric analysis for 4-hydroxyproline utilized a modification of the procedure of Cleary and Saunders.[21] NMR spectra were obtained at room temperature with a Jeolco 60-MHz instrument. Optical rotation was measured in a Bendix NPL polarimeter.

**Reaction Mixture.** Equal amounts of glyoxal trimeric dihydrate and oxaloacetic acid, usually 0.5 to 0.6 mmol, were dissolved in water; the solution was adjusted to pH 7.5 with 1 N NaOH, brought to a final volume of 10 mL, and kept at 20–25 °C for 24 h, then treated with 0.75 mL of concentrated $NH_4OH$. After 10 min, sodium borohydride (0.4 g) was added, and after a further 10 min, the reaction mixture was acidified to pH 1 (pH paper) with 6 N HCl. Aliquots of the reaction mixture, assayed for hydroxyproline, showed yields ranging from 26 to 41%.

**Estimation of *cis*- and *trans*-4-Hydroxyproline and Other Amino Acids.** After addition of [$^{14}$C]-*trans*-4-hydroxyproline as a tracer, an aliquot of the reaction mixture was desalted by passage through Dowex-50 $H^+$ (1 × 33 cm column) and elution with 0.23 N $NH_4OH$; recovery of both radioactively and chemically determined hydroxyproline was close to 100%. The $NH_3$ was removed by flash evaporation and the dry residue was taken up in dilute HCl (pH 1.5). Amino acid analysis showed equal amounts of *cis*- and *trans*-4-hydroxyproline as the major peaks. Small peaks in the positions of aspartic acid, serine, and alanine were also present, the peak consistent with aspartic acid (approximately one-sixth the molar quantity of hydroxyproline) being the major impurity. Oxaloacetic acid was apparently responsible for these side products, since its omission from the reaction mixture prevented their appearance, while the omission of glyoxal alone did not.

**Purification, Separation of Cis and Trans Racemates, and Crystallization.** A large-scale reaction mixture, identical with that described above, utilized 6 mmol each of glyoxal trimeric dihydrate and oxaloacetic acid. It was treated exactly as noted and yielded 1.7 mmol (28%) of hydroxyproline. [$^{14}$C]-*trans*-Hydroxyproline was added as a tracer, the reaction mixture was evaporated to dryness, and, to remove a large proportion of the salt present, the dry residue was extracted twice by stirring with 30 mL of 80% ethanol for 1 h at 5 °C. Ethanol was removed by flash evaporation; the residue was taken up in 10 mL of water and passed through a column of Dowex 1 acetate (AG-1 × 8, 3 × 25 cm) to remove aspartic acid. The effluent was evaporated to dryness, taken up in 5 mL of water, acidified to pH 2, applied to a Dowex 50 $H^+$ column (3 × 25 cm), and eluted with 0.23 N $NH_4OH$.

Separation of the cis and trans forms followed a scaled-up modification of the method of Piez et al.[22] utilizing a heated column (55 °C, 1.6 × 70 cm) of AG 50 W × 12, 400-mesh resin;[23] only one-third of the pooled eluates from the Dowex 50 column above was chromatographed at a time. In each run, *trans*-hydroxyproline, coincident with the radioactive tracer, was eluted as a separate peak (125–150 mL) followed closely by *cis*-hydroxyproline. Corresponding peaks were pooled from the three runs and represented 470 μmol of *trans*-hydroxyproline and 520 μmol of *cis*-hydroxyproline, about 60% recovery of the sample loaded. Each pool was evaporated to dryness and again desalted by adsorption to Dowex 50 $H^+$ as above and elution with 0.23 N $NH_4OH$. The eluates were pooled, dried, and dissolved with heating in a small volume of 90% ethanol. Crystals appeared after several days at 5 °C. On drying, these first crops weighed 30 (trans form) and 24 mg (cis form).

**Identity and Purity of the Crystalline Racemates.** Samples of each crystalline product were run on paper electrophoresis, on the amino acid analyzer, and from each, NMR spectra were obtained in $D_2O$. Amino acid analysis indicated that each sample was eluted in the expected position and was free of other ninhydrin-reactive peaks. Paper electrophoresis showed that each sample migrated with the authentic reference isomer. NMR spectra indicated identity with the corresponding reference standard, hydroxy-L-proline or allohydroxy-D-proline; the spectra agreed with those reported earlier.[25] As expected for this synthetic route, solutions of the trans and cis forms of hydroxyproline gave zero rotation, indicating each as the racemate.

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**Registry No.**—Glyoxal, 107-22-2; oxaloacetic acid, 32842-7; *cis*-4-hydroxyproline, 49761-17-3; *trans*-4-hydroxyproline, 618-28-0.

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## A Convenient Synthesis of 3- and 4-Methylphthalonitrile

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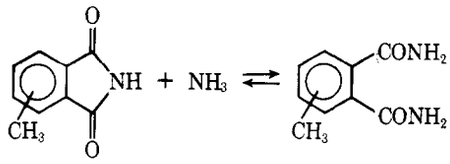
Recent interest in more soluble derivatives of N-donor chelating ligands, such as phthalocyanine[1,2] and 1,3-bis(2-pyridylimino)isoindoline,[3,4] made desirable a facile synthesis for precursor alkyl-substituted phthalonitriles. Although 3- and 4-methylphthalonitrile were first prepared many years ago, the multi-step synthesis is time consuming and deficient in overall yield.[5-8] More recently, alternate syntheses for alkyl-substituted phthalonitriles have been reported, but they rely upon less readily available starting materials.[9-11] We report here a convenient high yield synthesis of 3- and 4-methylphthalonitrile from the commercially available phthalic anhydrides. 3- and 4-alkylphthalic anhydrides are particularly suitable starting materials because alkyl-substituted phthalic anhydrides are obtainable from dehydrogenation of Diels–Alder adducts of maleic anhydride and the appropriately substituted butadienes.[12]

### Results and Discussion

Although unsubstituted phthalic anhydride may be readily converted to phthalonitrile via phthalimide and phthalamide intermediates, this chemical reaction sequence fails when applied to the synthesis of alkyl-substituted phthalonitriles. 3- and 4-methylphthalic anhydride **1** are readily converted to the corresponding imide **2** in high yield. However, unlike phthalimide itself, which reacts with ammonium hydroxide to form phthalamide in good yield, 3- and 4-methylphthalimide **2** react with ammonium hydroxide under identical conditions to form a water-soluble product characterized as the ammonium half-salt of the acid amide **4** (probably a mixture of the the two possible positional isomers). The infrared and elemental analyses are consistent with the presence of carboxylate and amide groups. Treatment of the salt **4** with thionyl chloride or heat resulted in reconversion to the starting imide **2**; treatment of salt **4** with dilute acid afforded the corresponding phthalic acid **5**.

Conversion of 3- or 4-methylphthalimide **2** to the diamide **3** was possible upon treatment of the imide with dry ammonia; up to 80% conversion was obtained and unreacted imide could be recycled. The difference in reactivity between unsubstituted phthalimide and 3- or 4-methylphthalimide **2** may be associated with the imide–amide equilibrium (eq 1), which is




(1)

apparently less favorable for 3- or 4-methylphthalamide than it is for unsubstituted phthalamide.

3-Methylphthalamide (**3**) was more easily converted to the nitrile **6** than was the 4-methyl derivative. With acetic anhydride the yield of 3-methylphthalonitrile was only 15%, but with $SOCl_2$/DMF at 0 or −12 °C the yield was as high as 80%. With acetic anhydride the yield of 4-methylphthalonitrile was considerably less than 15% and with $SOCl_2$/DMF at 0 °C the yield was still negligible (in the latter case the imide was the only major product observed). However, reducing the temperature of the $SOCl_2$/DMF reaction to −12 °C or using reverse addition resulted in yields of 4-methylphthalonitrile as high as 84%. The beneficial effect of lower temperatures on the $SOCl_2$/DMF dehydration of aromatic amides was reported earlier by Thurman.[13]

The convenient three-step sequence presented here allowed formation of 3-methylphthalonitrile in 60% and 4-methylphthalonitrile in 62% overall yield from commercially available starting materials.

### Experimental Section

3- and 4-methylphthalic anhydride were obtained from Eastman Chemicals and used as obtained. Infrared spectra were recorded from KBr pellets on a Perkin-Elmer Model 457 spectrophotometer; only pertinent absorption bands are reported. NMR spectra were recorded where solubility permitted with tetramethylsilane as an internal standard. Melting points are reported uncorrected. Microanalyses were performed by Central Laboratory Services of the Ford Motor Company.

**3-Methylphthalimide.** The imide was prepared from 3-methylphthalic anhydride according to the general procedure of Noyes and Potter.[14] The 3-methylphthalimide was conveniently purified by Soxhlet extraction with benzene and was obtained in 98% yield as white crystals, mp 186–187 °C (lit.[8] 189–190 °C).

**4-Methylphthalimide.** This imide was obtained by the same method as white crystals in 93% yield, mp 195–196 °C (lit.[6] 196 °C).

**Reaction of 3-Methylphthalimide with Ammonium Hydroxide.** A flask was charged with 0.22 g of 3-methylphthalimide, 5 mL of ethanol, and 8 mL of aqueous ammonia. The mixture was stirred at 24 °C for 40 h. After the solvent was evaporated under a stream of $N_2$, the residue was recrystalized from methanol–ethyl acetate to yield

Scheme I