crystallized from petroleum ether (28–38°) and had stood at −20° for 24 hours, 8 g. of crystals, m.p. 89–90°, was obtained.

Cyclization of 4.3 g. of this acid by means of a solution of 80 g. of phosphorus pentoxide in 50 cc. of phosphoric acid gave 3.5 g. of a neutral oil which could not be crystallized. A positive test with 2,4-dinitrophenylhydrazine was obtained. Clemmensen reduction of the ketone in the usual way and dehydrogenation over 30% palladium–charcoal[21] gave an oil which would not crystallize, and with picric acid, a picrate which was either too unstable or too impure for isolation.

β-5-Benzosuberylpropionic Acid (XI).—By concentration of the decanted mother liquors from the purification of the above acid (X), 2.4 g. of an oil soluble in aqueous sodium bicarbonate was obtained. Attempts to induce crystallization by cooling to −70° or standing at −20° and seeding with the crystalline acid, produced no solid acid. From 0.215 g. of the oil and 3 cc. of thionyl chloride with 30 minutes refluxing there was obtained the acid chloride which was added to 15 cc. of cold ammonia. The amide, recrystallized repeatedly from aqueous ethanol melted at 101.5–103°, 0.15 g.

Anal. Calcd. for $C_{14}H_{19}NO$: N, 6.45. Found: N, 6.45.

3-Keto-1,2,3,7,8,9,10,10a-octahydrocyclohepta[de]-naphthalene (XII).—A solution of 50 g. of phosphorus pentoxide in 32 cc. of phosphoric acid prepared as usual was poured into a flask containing 1.88 g. of XI. After 2 hours on the water-bath, the diluted reaction mixture was treated in the usual manner to give 1.6 g. of oil which crystallized from petroleum ether (28–38°) when cooled in a Dry Ice–acetone-bath, m.p. 43–44°.

Anal. Calcd. for $C_{14}H_{16}O$: C, 83.96; H, 8.05. Found: C, 84.18; H, 8.00.

The oxime prepared with hydroxylamine hydrochloride in absolute alcohol and pyridine melted at 151–152° after repeated crystallization from petroleum ether (60–70°).

Anal. Calcd. for $C_{14}H_{17}ON$: N, 6.51. Found: N, 6.66.

7,8,9,10-Tetrahydrocyclohepta[de]naphthalene (IV).—A 200-mg. portion of pale yellow oil obtained by Clemmensen reduction of the ketone (XII) was combined with 50 mg. of 30% palladium–charcoal[21] and heated for 90 minutes at 300° under nitrogen. After dissolving in benzene and separation from the catalyst, the residual oil was combined with 250 mg. of picric acid. The red picrate which formed at once, crystallized from alcohol, 60 mg., m.p. 100–109.5°. A second crop of 140 mg., m.p. 102.5–109°, was also obtained. By washing a benzene solution of the second crop with dilute ammonia and concentration of the benzene followed by sublimation at 1 mm. onto a cold finger, 45 mg. of colorless crystals m.p. 52–56° was obtained. These did not depress the m.p. of the material made by the first method.

Similarly a picrate of the sublimed material melted at 113.5–115° and was unchanged on mixing with the picrate obtained previously.

SALT LAKE CITY, UTAH RECEIVED SEPTEMBER 11, 1950

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING, UNIVERSITY OF CALIFORNIA]

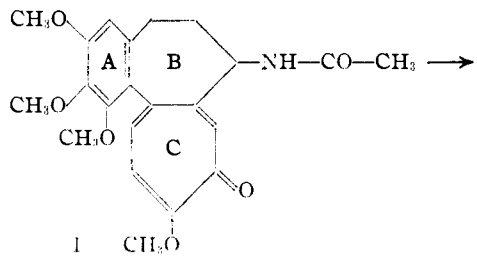
The Synthesis of DL-Colchinol Methyl Ether[1,2]

BY HENRY RAPOPORT, ARTHUR R. WILLIAMS AND MERLE E. CISNEY

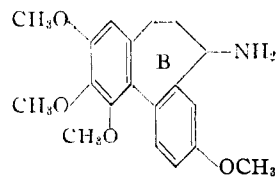
7-Amino-1,2,3,9-tetramethoxydibenzo[a,c][1,3]cycloheptadiene, prepared by an unambiguous synthesis from 2,3,4,7-tetramethoxy-10-phenanthroic acid, has been shown to be identical with racemized colchinol methyl ether. Thus the position of the amino group and the seven-membered nature of ring B in colchinol methyl ether have been established.

The action of the alkaloid colchicine (I) as a mitotic poison[3] has stimulated interest in it and related compounds as possible chemotherapeutic agents against cancer.[4] However, in order to study better the relationship of structure to activity and to synthesize truly analogous compounds, it is necessary to have a detailed knowledge of the structure of the parent compound. The objective of the present work has been to provide some such knowledge through the synthesis of colchinol methyl ether (II), an important degradation product. Since the reactions involved in the degradation may reasonably be assumed to have proceeded without rearrangement in ring B, proving the structure of colchinol methyl ether should establish the structure of colchicine except for ring C.

The structural features in question in colchinol methyl ether are the nature of ring B and the position of the amino group. Recent elegant



I



II

degradative work[5] has presented strong evidence that ring B is seven-membered. Assignment of the position of the amino group is based on the isolation of 4-methoxyphthalimide from the chromic oxide oxidation of N-acetylcolchinol methyl ether.[6] We wish to report the confirmation of both these

(1) This work was supported in part by a grant from the Cancer Research Committee, University of California.

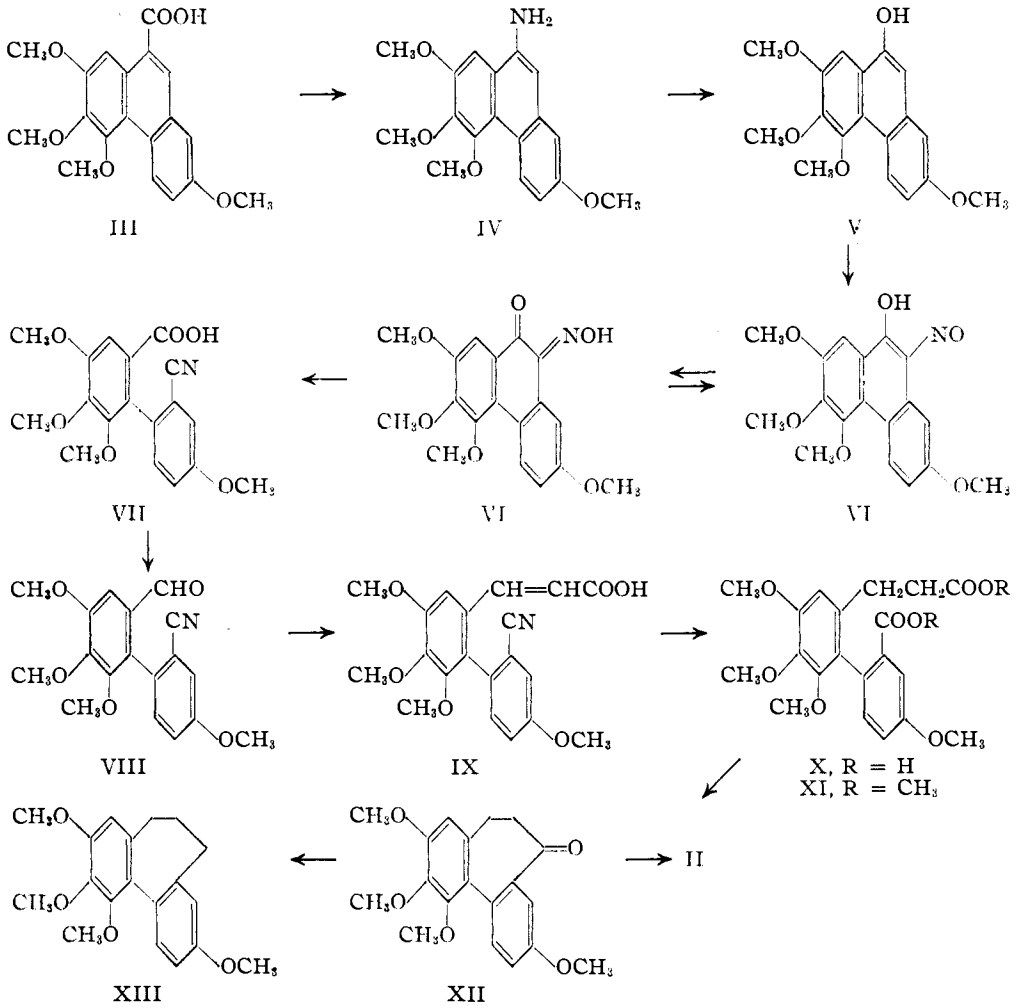
(2) Presented in part before the Division of Organic Chemistry, American Chemical Society, Philadelphia Meeting, April 11, 1950. A preliminary report of this work appeared as a Communication to the Editor, THIS JOURNAL, **72**, 3324 (1950).

(3) (a) J. M. Krythe and S. J. Wellensiek, *Bibliogr. genet.*, **14**, 1 (1942); (b) M. Levine, *Botan. Rev.*, **11**, 145 (1945); (c) R. J. Ludford, *J. Natl. Cancer Inst.*, **6**, 89 (1945).

(4) (a) H. Lettre and H. Fernholz, *Z. physiol. Chem.*, **278**, 175 (1943); (b) B. Goldberg, L. G. Ortega, A. Golden, G. E. Ullyot and E. B. Schoenbach, *Cancer*, **3**, 124 (1950).

(5) (a) G. L. Buchanan, J. W. Cook and J. D. Loudon, *J. Chem. Soc.*, 325 (1944); (b) N. Barton, J. W. Cook and J. D. Loudon, *ibid.*, 176 (1945); (c) D. S. Tarbell, H. R. Frank and P. E. Fanta, THIS JOURNAL, **68**, 502 (1946).

(6) A. Windaus, *Ann.*, **439**, 59 (1924).



structural proposals by the synthesis of *dl*-colchinol methyl ether.[7]

The synthetic procedure paralleled for the most part the recently developed general method for preparing dibenzocycloheptadienes.[8] As starting material, the required 2,3,4,7-tetramethoxy-10-phenanthroic acid (III) was synthesized by a method more suited for large scale preparation than that previously reported.[5b] 3,4,5-Trimethoxyphenylacetic acid, prepared by the single-step reduction and hydrolysis of the aldehyde cyanohydrin,[9] was condensed with 2-nitro-5-methoxybenzaldehyde, and the resulting nitrocinnamic acid reduced to the amino acid. The convenient procedure of Cassaday and Bogert[10] was then used to effect ring-closure to the substituted phenanthroic acid.

Two paths were considered for converting the phenanthroic acid (III) to the ring-opened cyano-acid (VII). One was oxidation to the quinone followed by monoxime formation and ring-opening by means of the Beckman rearrangement. Since the quinone in this case (2,3,4,7-tetramethoxyphenanthrenequinone) is unsymmetrical, a mixture of monoximes was anticipated and it was intended to separate isomers at the cyano-acid stage. The desired isomer should be distinguishable through its ability to form a fluorenone. However, the low yields of quinone realized from oxidation of the phenanthroic acid (III), the phenanthrene (prepared by decarboxylation), or the phenanthrylamine (IV) led to the abandonment of this method.

Instead, the phenanthrylamine, prepared from the acid by Curtius degradation, was converted to the phenanthrol (V) and this in turn was nitrosated to give the nitrosophenanthrol, tautomeric with the quinonemonoxime. By this procedure a single, specific monoxime of an unsymmetrical phenanthrenequinone may be easily prepared from the corresponding acid. In the present case, 2,3,4,7 - tetramethoxyphenanthrenequinone - 9 - monoxime (VI) was obtained in 64% over-all yield from 2,3,4,7-tetramethoxy-10-phenanthroic acid (III). Beckmann rearrangement then proceeded readily, resulting in the necessary cyano-acid (VII).

Difficulty was anticipated in the next step—the preparation of the acid chloride of VII—because

(7) G. L. Buchanan, J. W. Cook, J. D. Loudon and J. MacMillan, *Nature*, **162**, 692 (1948), in a preliminary communication, reported the synthesis of a compound identical with one of the oxidation products of deaminocolchinol methyl ether. However, no experimental details have as yet appeared. In another preliminary account, which appeared after our original communication on the synthesis of *dl*-colchinol methyl ether (ref. 2), J. W. Cook, J. Jack and J. D. Loudon [*Chemistry and Industry*, 650 (1950)] have reported another synthesis which is in good agreement with and similar to ours in the later stages.

(8) H. Rapoport and A. R. Williams, THIS JOURNAL, **71**, 1774 (1949).

(9) J. Levine, T. E. Eble and H. Fishback, *ibid.*, **70**, 1930 (1948).

(10) J. T. Cassaday and M. T. Bogert, *ibid.*, **61**, 2461 (1939).

of the expected concomitant fluorenone formation. In the case where there were no methoxyl groups present, preparation of the acid chloride was accompanied by a small amount (<10%) of the fluorenone,[8] whereas attempts to prepare the acid chloride of 2-(4′-methoxyphenyl)-3,4,5-trimethoxybenzoic acid (compound VII without the cyano group) using either phosphorus pentachloride[11] or thionyl chloride[12] resulted exclusively in fluorenone formation. Surprisingly, however, in the present case, with both cyano and methoxyl groups present, the cyano-acid (VII) was converted to acid chloride and thence to cyano-aldehyde (VIII) by Rosenmund reduction without the formation of any detectable amount of fluorenone.

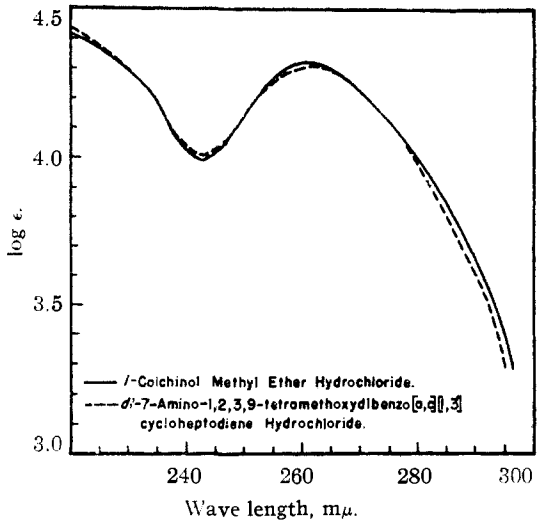


Fig. 1.—Ultraviolet absorption spectra: Cary spectrophotometer, 3×10^{-5} M solutions in methanol.

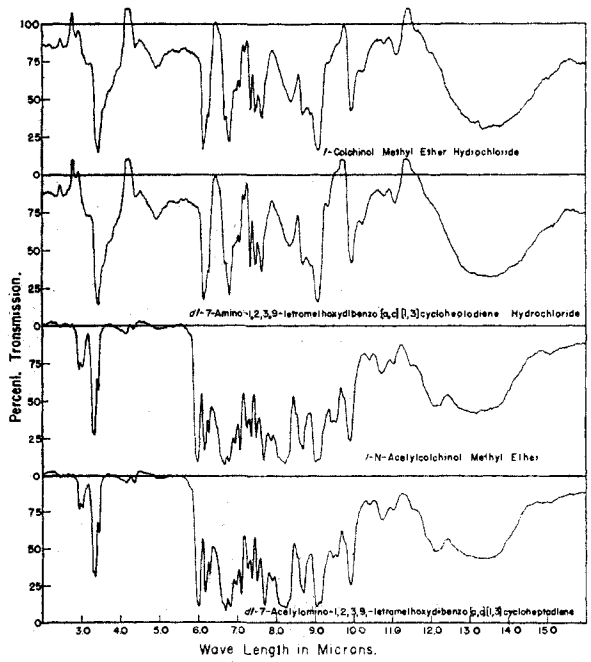


Fig. 2.—Infrared absorption spectra: Baird spectrophotometer with NaCl prism, 1.5% solution of the hydrochlorides and 13.5% solutions of the acetyl derivatives in chloroform.

Of the various procedures for cyclization to the ketone (XII), Dieckmann condensation of the dimethyl ester (XI) proved to be by far the most efficacious. However, the conditions found satisfactory in the non-methoxylated series[8] (sodium and a 2-hour heating period) were ineffective and a more drastic procedure (potassium and 11 hours of heating) had to be employed. The resulting crude β-ketoester was then hydrolyzed and decarboxylated to give the ketone in 69% yield. From the tetramethoxyphenanthroic acid (III) to 1,2,3,9-tetramethoxydibenzo[a,c][1,3]cycloheptadiene-7-one (XII), the over-all yield was 17%.

When this ketone was subjected to Wolff–Kishner reduction, 1,2,3,9-tetramethoxydibenzo[a,c][1,3]cycloheptadiene (XIII) was obtained and proved to be identical with dihydrodeaminocolchinol methyl ether. Thus there is no question about the presence of a seven-membered ring in the latter compound, and although it is reasonable to assume that degradation of colchinol methyl ether to the deamino compound followed by hydrogenation involved no rearrangement,[13] the identity of compound XIII and dihydrodeaminocolchinol methyl ether does not provide the desired unequivocal evidence for the seven-membered ring in colchinol methyl ether itself.

To secure a compound with the structure proposed for colchinol methyl ether (II), the oxime of the ketone (XII) was reduced catalytically to 7-amino-1,2,3,9-tetramethoxydibenzo[a,c][1,3]-cycloheptadiene (II). This resulted, of course, in optically inactive material, and in order to establish the identity of the synthetic and natural products it became necessary to either resolve the former or racemize the latter.

However, before exploring these alternatives, a spectral comparison of the natural (*l*) and synthetic (*dl*) compounds was considered desirable. Both materials should have identical ultraviolet and infrared absorption spectra, in solution, and the detailed structure of the latter should be especially useful for demonstrating identity. The ultraviolet absorption spectra[14a] of the hydrochlorides (Fig. 1) and the N-acetyl derivatives,[14b] and the

(11) H. R. Frank, P. E. Fanta and D. S. Tarbell, THIS JOURNAL, **70**, 2314 (1948).

(12) N. Barton, J. W. Cook, J. D. Loudon and J. MacMillan, *J. Chem. Soc.*, 1079 (1949).

(13) The procedure used, *viz.*, heating N-acetylcolchinol methyl ether with phosphorus pentoxide in boiling xylene (ref. 36), is certainly open to question. However, the same deamino compound is obtained from colchinol methyl ether by Hofmann deamination (ref. 5a), which apparently proceeds without rearrangement in the carbon skeleton [P. G. Stephens and J. H. Richmond, THIS JOURNAL, **63**, 3132 (1941)].

(14) (a) We are indebted to Dr. D. S. McClure for the ultraviolet spectra and to Dr. N. K. Freeman for the infrared. (b) These were identical with the curve for N-acetylcholchinol methyl ether reported by H. T. Huang, D. S. Tarbell, and H. R. V. Arnstein, *ibid.*, **70**, 4181 (1948).

infrared spectra of these derivatives (Fig. 2) were found to be identical. On this basis, structure II may be considered as established for colchinol methyl ether. However, since the effect of a minor variation in structure (such as changing the amino group to position 6 or 5[15]) on the spectra of such compounds is not known with certainty, further evidence was sought before concluding the structure proved.

Attempts were made to resolve the synthetic material with tartaric, malic, mandelic, α-bromo-π-camphorsulfonic and 10-camphorsulfonic acids. In those cases where crystalline salts were obtained (with *d*-tartaric and *l*-malic acids), the salt of the *dl*-amine was less soluble and higher melting than that of the *l*-amine and no resolution could be effected.

Racemization of colchinol methyl ether was then examined and found to proceed with facility when the N-benzylidene derivative was heated with methanolic benzyltrimethylammonium hydroxide. The *dl*-cholchinol methyl ether isolated after acid hydrolysis formed a hydrochloride and N-acetyl derivative identical with the corresponding synthetic compounds. Since the synthesis was unambiguous, colchinol methyl ether has been thus proved to have structure II.

Experimental[16]

2-Nitro-5-methoxybenzaldehyde.—Commercial *m*-nitrobenzaldehyde was converted to ***m*-hydroxybenzaldehyde**[17] and thence to **2-nitro-5-hydroxybenzaldehyde** by nitration of the carbonate.[18]

Methylation by the procedure used for preparing veratraldehyde[19] gave 2-nitro-5-methoxybenzaldehyde, m.p. 82–83° (reported[18] m.p. 83–84°), in a 25% over-all yield of pure material.

3,4,5-Trimethoxybenzaldehyde.—Trimethoxybenzoic acid, prepared by methylation of gallic acid,[20] was converted to the aldehyde *via* Rosenmund reduction of the acid chloride. The acid chloride was prepared from the acid in a benzene solution by treatment with 250 mole % of thionyl chloride. Distillation of the reaction mixture gave a 91% yield of **3,4,5-trimethoxybenzoyl chloride**, b.p. 155–160° (2.5 mm.); after crystallization from benzene–pentane, m.p. 77–79° (reported[21] b.p. 185° (18 mm.), m.p. 77–78°).

The acid chloride was reduced immediately after distillation using palladium–barium sulfate and a sulfur–quinoline poison.[22] The aldehyde was best isolated through the bisulfite addition compound[23] in an 81% yield.

3,4,5-Trimethoxyphenylacetic Acid.—3,4,5-Trimethoxybenzaldehyde by a procedure analogous to that used by Levine, Eble and Fishback[9] for the preparation of *o*-methoxyphenylacetic acid. The chief changes were omission of the hydriodic acid and modification of the isolation procedure since the 3,4,5-trimethoxyphenylacetic acid is insoluble in carbon tetrachloride, the extractant used in the original method.

To prepare the cyanohydrin, a solution of 53.5 g. (0.52 mole) of sodium bisulfite in 194 ml. of water was added to 74.6 g. (0.38 mole) of 3,4,5-trimethoxybenzaldehyde and the mixture was stirred until the bisulfite addition product formed, usually within 10 minutes. Sufficient water to form a thin paste was then added followed by 600 ml. of ether and the mixture was cooled in an ice-bath. To this mixture, with stirring, was now added in one portion an ice-cold solution of 46.6 g. (0.95 mole) of sodium cyanide in 114 ml. of water. After one-half hour, 5.5 g. of sodium bisulfite was added, and stirring was continued for 1.5 hours, after which the layers were separated and the aqueous phase extracted with two 150-ml. portions of ether. The combined ether extracts were washed with two 150-ml. portions of 20% sodium bisulfite solution and two 150-ml. portions of water, and the ether was then evaporated. The residue was dissolved in 375 ml. of benzene, the solution dried by azeotropic distillation, and petroleum ether (b.p. 30–60°) added to cloudiness. After cooling, the product was filtered, washed with a small amount of a mixture of equal parts of benzene and petroleum ether followed by petroleum ether, and dried; yield 82 g., 97%, of 3,4,5-trimethoxybenzaldehyde cyanohydrin, m.p. 99.6–100.8° (reported[24] m.p. 101°).

To 74.0 g. (0.33 mole) of cyanohydrin was added a hot solution of 108 g. (0.47 mole) of stannous chloride dihydrate in 108 ml. of glacial acetic acid and 108 ml. of concentrated hydrochloric acid. With stirring and in a nitrogen atmosphere, the mixture was heated in a boiling water-bath for 3 hours after which it was filtered hot, using 40 ml. of hot glacial acetic acid as a wash. The filtrate was concentrated at 15 mm. and a bath temperature 50° to one-fourth its volume, and the concentrate was poured slowly with stirring into one liter of a saturated sodium chloride solution. Cooling overnight gave the crude acid which was recrystallized from one liter of water to give 45 g. of the pure acid. By concentration of the mother liquors, an additional 9 g. was obtained, bringing the total yield of 3,4,5-trimethoxyphenylacetic acid to 54 g. (72%), m.p. 117–118° (reported[25] m.p. 119–120°).

2-Nitro-5-methoxy-α-(3′,4′,5′-trimethoxyphenyl)-cinnamic Acid.—This condensation was carried out according to the general direction of Bakunin and Peccerillo.[26] A mixture of 38.5 g. (0.17 mole) of 3,4,5-trimethoxyphenylacetic acid, 33.9 g. (0.19 mole) of 2-nitro-5-methoxybenzaldehyde, 52.3 g. (0.50 mole) of acetic anhydride, and 17.2 g. (0.17 mole) of triethylamine was heated at 90–95° for 16 hours after which 10 ml. of water was added at room temperature to decompose the excess acetic anhydride. After an hour, the reaction mixture was poured into a solution of 160 g. of potassium carbonate in 1.1 liters of water and the resulting mixture heated on the steam-bath to aid solution of the tarry material that had separated. The cooled solution was washed with two 200-ml. portions of benzene followed by three 200-ml. portions of ether, treated with decolorizing carbon, filtered, and cautiously acidified with concentrated hydrochloric acid. After thorough cooling, the precipitate was filtered, washed with cold water, and crystallized from 200 ml. of methanol to give 55.6 g. (84%) of the cinnamic acid, m.p. 157–159° (reported[5b] m.p. 157–159°).

2-Amino-5-methoxy-α-(3′,4′,5′-trimethoxyphenyl)-cinnamic Acid.—The method of Sharp,[27] developed for an analogous compound, proved the best and most consistent for reducing the nitro group. A solution of ferrous sulfate was prepared by adding 120 g. (0.71 mole) of the heptahydrate to 315 ml. of concentrated aqueous ammonia and 370 ml. of water at 75°. To this solution, stirred and maintained at 75°, was first added several drops of octanol and then, in a thin stream, a warm solution of 17.7 g. (0.046 mole) of the nitrocinnamic acid in 60 ml. of a mixture of equal volumes of water and concentrated aqueous ammonia. After an hour at 75°, the precipitated iron hydroxide was allowed to settle and the supernatant liquid was filtered through a mat of filter aid using four 100-ml. portions of

(15) It is hoped that information on this point may soon be obtained after completion of the synthesis of isocolchinol methyl ether (5-amino) and pseudocolchinol methyl ether (6-amino) now in progress.

(16) All melting points are corrected; microanalyses were performed by the Microchemical Laboratory, University of California.

(17) R. B. Woodward, "Organic Syntheses," Vol. 25, John Wiley and Sons, Inc., New York, N. Y., 1945, p. 55.

(18) F. A. Mason, *J. Chem. Soc.*, **127**, 1195 (1925).

(19) J. S. Buck, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 619.

(20) F. Mauthner, "Organic Syntheses," Coll. Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1941, p. 537.

(21) W. H. Perkin and C. Weizmann, *J. Chem. Soc.*, **89**, 1649 (1906).

(22) E. B. Hershberg and J. Cason, "Organic Syntheses," Vol. 21, John Wiley and Sons, Inc., New York, N. Y., 1941, p. 84.

(23) H. T. Huang, D. S. Tarbell and H. R. V. Arnstein, THIS JOURNAL, **70**, 4181 (1948).

(24) K. H. Slotta and G. Haberland, *J. prakt. Chem.*, **139**, 211 (1934).

(25) F. Mauthner, *Ber.*, **41**, 3662 (1908).

(26) M. Bakunin and D. Peccerillo, *Gazz. chim. ital.*, **65**, 1145 (1935).

(27) T. M. Sharp, *J. Chem. Soc.*, 1234 (1936).

dilute aqueous ammonia as wash. The cooled filtrate was acidified to *p*H 3.3 with concentrated hydrochloric acid, and the precipitate was recrystallized from 95% ethanol to give 14.1 g. (87%) of amino acid, m.p. 189–191° (reported[5b] m.p. 189–190°).

2,3,4,7-Tetramethoxy-10-phenanthroic Acid (III).—The Pschorr ring-closure procedure of Cassaday and Bogert[10] was used for the preparation of this acid. To a solution of 35.3 g. (0.098 mole) of 2-amino-5-methoxy-α-(3′,4′,5′-trimethoxyphenyl)-cinnamic acid in 700 ml. of dioxane was slowly added, with vigorous stirring, 7.7 ml. of concentrated sulfuric acid. After the mixture, which had become pasty, had cooled to room temperature, 16.3 g. (0.14 mole) of isoamyl nitrite was added and stirring continued for one hour. The suspension of diazonium salt was then added in aliquots during one-half hour to a stirred mixture of 200 ml. of 6.8 molar sodium hypophosphite and 6 g. of Gattermann copper paste[28] maintained at 50–60°. After the addition was completed and nitrogen evolution had ceased, the mixture gave a negative coupling test with β-naphthol and was then poured into four liters of water containing 80 ml. of concentrated aqueous ammonia. Treatment with carbon and filtration followed by acidification of the filtrate with concentrated hydrochloric acid gave the crude phenanthroic acid which was crystallized from ethanol; yield 21.1 g. (63%), m.p. 201–202° (reported[5b] m.p. 200°).

A further 3% could be obtained as methyl ester by evaporating the mother liquors to dryness and heating the residue under reflux overnight with 3% methanolic sulfuric acid. The ester was isolated in the usual manner and distilled at 300–325° (bath temperature) at 1 mm. Crystallization from methanol gave material melting at 104–105° (reported[5b] m.p. 103–104°).

2,3,4,7-Tetramethoxyphenanthrene.—Decarboxylation of the phenanthroic acid was achieved using a modification of the method of Galimberti.[29] A mixture of 1.9 g. (5.5 millimoles) of acid (III), 16 ml. of quinoline and 0.1 g. of copper sulfate was heated at 200–210° until carbon dioxide evolution ceased (3 hours). After cooling and adding 120 ml. of benzene, the reaction mixture was filtered and the filtrate washed with 1 *N* hydrochloric acid, 1 *N* potassium carbonate, and water. Evaporation of the benzene followed by sublimation at reduced pressure and crystallization of the sublimate from absolute ethanol gave 1.45 g., 86% yield, of the tetramethoxyphenanthrene, m.p. 151–152°.

Anal. Calcd. for $C_{18}H_{18}O_4$: C, 72.5; H, 6.1; OCH_3, 41.6. Found: C, 72.6; H, 6.0; OCH_3, 41.8.

The picrate was formed by heating under reflux for one hour an alcoholic solution of 2,3,4,7-tetramethoxyphenanthrene and picric acid. On cooling, the picrate crystallized as deep red needles which were filtered and washed with cold alcohol followed by ether; m.p. 123–123.5°.

Anal. Calcd. for $C_{24}H_{22}O_{11}N_3$: C, 54.7; H, 4.0; N, 8.0. Found: C, 54.7; H, 4.0; N, 8.1.

2,3,4,7-Tetramethoxy-10-phenanthrylamine (IV).—A solution of 25 g. (0.073 mole) of the phenanthroic acid (III), 18.3 g. (0.15 mole) of thionyl chloride, and 300 ml. of dry benzene was heated under reflux for one hour, then concentrated to 50 ml. on a steam-bath under a stream of dry nitrogen. Dry benzene (100 ml.) was added and the solution was again concentrated to 50 ml., this addition and concentration being repeated twice more. The final volume was adjusted to 100 ml. and the solution cooled to obtain the acid chloride; yield 25.4 g. (92%), m.p. 160–162°. Recrystallization of a sample from benzene gave material melting at 162–164°.

This acid chloride was dissolved in two liters of acetone, cooled in an ice-bath, and 4.8 g. (0.074 mole) of activated sodium azide[30] in 18 ml. of water was added with shaking. The solution was allowed to stand for 15 minutes, then six liters of ice-cold water was gradually added over a half-hour period and the precipitated azide was filtered and dried overnight in a vacuum desiccator. The crude azide and 300 ml. of absolute ethanol were heated under reflux for one hour, 300 ml. of 4 *N* ethanolic potassium hydroxide was added and refluxing was continued for 4 hours. After filtering, the hot reaction mixture was cooled to precipitate the amine, m.p. 153–154°. An additional portion could be obtained from the mother liquors by adding 400 ml. of water, evaporating the ethanol on the steam-bath with an air stream, and crystallizing the resulting precipitate from absolute ethanol; total yield, 19.2 g., 91% based on acid chloride or 84% based on phenanthroic acid. An analytical sample of the amine was prepared by several recrystallizations from absolute ethanol; m.p. 153.5–154°.

Anal. Calcd. for $C_{18}H_{19}O_4$: C, 69.0; H, 6.1; N, 4.5. Found: C, 69.0; H, 6.2; N, 4.2.

In a run where the yield of amine from acid chloride was lower (81%) due to excessive water being present during urethan formation, a 6.5% yield of **1,3-bis-(2,3,4,7-tetramethoxy-10-phenanthryl)-urea** was isolated. It is insoluble in absolute ethanol and was purified for analysis by crystallization from ethanol–chloroform; 1.3 g. required 200 ml. of chloroform and 100 ml. of absolute ethanol; m.p. 277.6–278°.

Anal. Calcd. for $C_{37}H_{36}N_2O_9$: C, 68.1; H, 5.6; N, 4.3. Found: C, 68.2; H, 5.6; N, 4.4.

Hydrolysis of this symmetrical urea with potassium hydroxide in ethylene glycol at 150° for 18 hours gave a 64% recovery of amine upon diluting the hydrolysate with water and crystallizing the precipitate from ethanol.

N-Acetyl-2,3,4,7-tetramethoxy-10-phenanthrylamine was prepared by heating the amine on the steam-bath for 5 minutes with a twofold excess of acetic anhydride. Excess acetic anhydride was decomposed with water, and the precipitated N-acetyl derivative was crystallized from ethanol, m.p. 226–228°.

Anal. Calcd. for $C_{20}H_{21}NO_5$: C, 67.6; H, 6.0; N, 3.9. Found: C, 67.3; H, 5.9; N, 3.8.

2,3,4,7-Tetramethoxyphenanthrenequinone.—Oxidation of the phenanthroic acid (III) was carried out as previously reported[5b] except that heating was confined to the steam-bath instead of under reflux. From 1.20 g. of acid there was recovered 0.53 g. of starting material and 0.12 g. (19% yield) of quinone, m.p. 195–196° (reported[5b] m.p. 193–194°), after purification through the bisulfite addition product.

Oxidation of 2,3,4,7-tetramethoxyphenanthrene gave only traces of quinone, and oxidation of the phenanthryl amine (IV) afforded quinone in 24% yield.

2,3,4,7-Tetramethoxy-10-phenanthrol (V).—The procedure followed for the conversion of the phenanthrylamine to the phenanthrol was a modification of that of MacDonald and Chechak.[31] A solution of 20.0 g. (0.064 mole) of amine (IV) in 200 ml. of dioxane and 200 ml. of water was saturated with sulfur dioxide at 0°, then heated in sealed tubes at 100–105° for 48 hours. After cooling, the bomb tubes were opened, the contents were poured into three liters of water, and the sulfur dioxide was allowed to evolve as the phenanthrol precipitated. The solid was digested on the steam-bath for one hour with one liter of 1 *N* NaOH, filtered from a small amount of insoluble material, and the filtrate acidified with concentrated hydrochloric acid. The precipitated phenanthrol was crystallized from aqueous ethanol; yield 18.5 g. (92%), m.p. 167–169°.

Anal. Calcd. for $C_{18}H_{18}O_5$: C, 68.8; H, 5.8. Found: C, 68.7; H, 5.9.

The methyl ether, **2,3,4,7,10-pentamethoxyphenanthrene**, was prepared by shaking a solution of 0.5 g. of the phenanthrol in 35 ml. of 3 *N* NaOH for 2 hours at room temperature with 5 ml. of dimethyl sulfate. The pentamethoxyphenanthrene separated as a light pink solid which was purified by crystallizing from methanol and then distilling onto a cold finger at 150° (bath temperature) at 0.05 mm.; m.p. 111–112°.

Anal. Calcd. for $C_{19}H_{20}O_5$: OCH_3, 47.3. Found: OCH_3, 47.4.

2,3,4,7-Tetramethoxyphenanthrenequinone-9-oxime (VI).—A solution of 19.7 g. (0.063 mole) of phenanthrol (V) in 90 ml. of 1 *N* potassium hydroxide to which was added 5 g. (0.072 mole) of sodium nitrite in 40 ml. of water was cooled to 0° and, with stirring, 240 ml. of 1 *N* sulfuric acid was added during an hour, keeping the solution at 3–5°. Stirring was continued for one-half hour, the mixture was

(28) L. Gattermann, *Ber.*, **23**, 1218 (1890).

(29) L. Galimberti, *Bull. sci. facolta chim. ind. Bologa*, 351 (1940); C. A., **37**, 3410[1] (1943).

(30) P. A. S. Smith, "Organic Reactions," Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1946, p. 382.

(31) S. F. MacDonald and A. J. Chechak, THIS JOURNAL, **70**, 1972 (1948).

filtered, and the precipitate was washed with water until the washings were neutral. The red monoxime was crystallized from absolute alcohol; yield 18.0 g. (84%), m.p. 154–155° followed by solidification on further heating and remelting at 173–175°.

Anal. Calcd. for $C_{18}H_{17}NO_6$: C, 63.0; H, 5.0; N, 4.1. Found: C, 63.0; H, 5.0; N, 4.1.

2-(2′-Cyano-4′-methoxyphenyl)-3,4,5-trimethoxybenzoic Acid (VII).—A solution of 20.0 g. (0.058 mole) of the monoxime (VI), 11.4 g. (0.065 mole) of benzenesulfonyl chloride, and 160 ml. of pyridine was heated under reflux with stirring for 1.5 hours. The cooled solution was added to 800 ml. of 6 *N* HCl, cooled in an ice-bath, and the red solid which precipitated was filtered, washed well with water, and dissolved in 500 ml. of methanol. To the methanol solution was added 100 ml. of 2 *N* potassium carbonate solution, and the resulting solution was heated under reflux for one hour. Then 100 ml. of water was added, the methanol was evaporated on the steam-bath with an air stream, and the solution was filtered after treating with decolorizing carbon. Acidification gave a precipitate of the cyano acid which was crystallized from benzene (150 ml.)–hexane (15 ml.); yield 15.2 g. (76%), m.p. 216.5–218°.

Anal. Calcd. for $C_{18}H_{17}NO_6$: C, 63.0; H, 5.0; N, 4.1; eq. wt., 343. Found: C, 63.0; H, 5.1; N, 3.9; eq. wt., 341.

Hydrolysis of the cyano acid was effected by boiling 0.3 g. with 1 g. of potassium hydroxide in 50 ml. of water for 24 hours. Treating with carbon, filtering, and acidifying gave 0.24 g. (76%) of **2-(2′-carboxy-4′-methoxyphenyl)-3,4,5-trimethoxybenzoic acid**, m.p. 240–241° (reported[12] m.p. 240–241°).

2-(2′-Cyano-4′-methoxyphenyl)-3,4,5-trimethoxybenzaldehyde (VIII).—The acid chloride was prepared from 10.6 g. (0.031 mole) of cyano acid (VII), 20 g. (0.17 mole) of thionyl chloride and 150 ml. of benzene by heating under reflux for one hour. The benzene and thionyl chloride were evaporated on a steam-bath with a dry nitrogen stream, the residue was dissolved in 100 ml. of benzene, and the solution again evaporated to dryness. This process was repeated two more times, and the final residue was dissolved in 150 ml. of anhydrous xylene to which was added 2.2 g. of 5% palladium-on-barium sulfate and 0.2 ml. of sulfur–quinoline poison. Reduction was carried out according to the general procedure[22] and was complete in 4 hours. The solution was filtered, the xylene removed under reduced pressure, and the residue, after being dissolved in 300 ml. of methanol, was heated under reflux for one hour with 100 g. of 40% sodium bisulfite solution. The methanol was then evaporated using an air stream and the solution diluted with 100 ml. of water and filtered hot. This bisulfite digestion of the insoluble material was repeated six times, and the bisulfite addition compound in the combined extracts was decomposed with saturated sodium carbonate solution. The aldehyde slowly precipitated and was crystallized from aqueous ethanol; yield 6.8 g. (67%), m.p. 92–92.5° after several recrystallizations.

Anal. Calcd. for $C_{18}H_{17}NO_5$: C, 66.1; H, 5.2. Found: C, 65.8; H, 5.2.

The semicarbazone was prepared by heating under reflux for 5 minutes an aqueous alcoholic solution of the aldehyde, semicarbazide hydrochloride, and sodium acetate. The semicarbazone crystallized on cooling and was recrystallized from aqueous ethanol, m.p. 222–223°.

Anal. Calcd. for $C_{19}H_{20}N_4O_5$: N, 14.6. Found: N, 14.4.

2-(2′-Cyano-4′-methoxyphenyl)-3,4,5-trimethoxycinnamic Acid (IX).—A solution of 12.9 g. (0.039 mole) of the cyano-aldehyde (VIII), 8.2 g. (0.079 mole) of malonic acid, 40 ml. of pyridine, and 0.8 ml. of piperidine was heated at 80° for one-half hour, 100° for 2 hours, and under reflux for an additional half hour. The cooled solution was slowly poured into 200 ml. of 6 *N* HCl, cooled in an ice-bath, and the resulting precipitate was filtered, washed with water, and digested for one hour with 150 ml. of boiling 1 *N* potassium carbonate. At the end of the digestion solution was complete and after treating with carbon and filtering hot, the filtrate was acidified with concentrated hydrochloric acid. The precipitated cyano-cinnamic acid was crystallized from aqueous ethanol; yield 13.5 g. (92%), m.p. 224–225°.

Anal. Calcd. for $C_{20}H_{19}NO_6$: C, 65.0; H, 5.2; eq. wt., 369. Found: C, 65.0; H, 5.3; eq. wt., 371.

The cyano-cinnamic acid could be converted to **β-[2-(2′-cyano-4′-methoxyphenyl)-3,4,5-trimethoxyphenyl]-propionic acid** by hydrogenating an aqueous solution of the sodium salt with a palladium–carbon catalyst. Acidification of the filtered solution gave the saturated acid which was crystallized from aqueous ethanol, m.p. 124.5–125.5°.

Anal. Calcd. for $C_{20}H_{21}NO_6$: C, 64.7; H, 5.7. Found: C, 64.7; H, 5.7.

β-[2-(2′-Cyano-4′-methoxyphenyl)-3,4,5-trimethoxyphenyl]-propionamide was prepared from the cyano-propionic acid by treating the acid chloride, obtained from the acid and thionyl chloride in benzene solution, with concentrated aqueous ammonia, and was crystallized from benzene, m.p. 170–171°.

Anal. Calcd. for $C_{20}H_{22}N_2O_5$: C, 64.9; H, 6.0; N, 7.6. Found: C, 65.0; H, 6.0; N, 7.5.

β-[2-(2′-Carboxy-4′-methoxyphenyl)-3,4,5-trimethoxyphenyl]-propionic Acid (X).—This dibasic acid was most conveniently prepared by hydrogenation and hydrolysis of the cyano-cinnamic acid (IX) without isolating the intermediate. A mixture of 6 g. (0.0163 mole) of the cyano-cinnamic acid, 17 ml. of 1 *N* NaOH, and 40 ml. of water was heated on the steam-bath until solution was complete, cooled to room temperature and hydrogenated at room temperature and atmospheric pressure with 1.2 g. of 5% palladium–carbon. When hydrogen absorption ceased in 30 minutes at 106% of the theoretical for one double bond, the solution was filtered from the catalyst, 17 g. of potassium hydroxide in 150 ml. of water was added, and the solution was heated under reflux for 24 hours. The solution was then filtered, cooled, and slowly acidified with 1 *N* HCl to precipitate the saturated dibasic acid crystalline. It was recrystallized from 20 ml. of methanol and 40 ml. of water to give 5.3 g. (84%), m.p. 175–176.5°.

Anal. Calcd. for $C_{20}H_{22}O_8$: C, 61.5; H, 5.7; eq. wt., 194. Found: C, 61.4; H, 5.9; eq. wt., 195.

Methyl β-[2-(2′-Carbomethoxy-4′-methoxyphenyl)-3,4,5-trimethoxyphenyl]-propionate (XI).—A solution of 4.5 g. (0.12 mole) of the saturated dibasic acid (X), 35 ml. of methanol and 1.5 ml. of concentrated sulfuric acid was heated under reflux for 18 hours. The cooled solution was diluted with 350 ml. of water and extracted with three 25-ml. portions of chloroform which were combined and washed with 30 ml. of 1 *N* sodium carbonate and 30 ml. of water, dried over magnesium sulfate, and the chloroform evaporated on the steam-bath. The residue was crystallized from aqueous methanol giving 4.6 g. (96%) of the dimethyl ester, m.p. 110–111°. For analysis, a sample was further purified by a short path distillation at 170° (bath temperature) at 0.05 mm.

Anal. Calcd. for $C_{22}H_{26}O_8$: OCH_3, 44.5. Found: OCH_3, 44.2.

1,2,3,9-Tetramethoxydibenzo[a,c][1,3]cycloheptadiene-7-one (XII).—To a stirred mixture of 1.24 g. (0.032 mole) of potassium, 10 ml. of toluene and 5 drops of methanol, heated to reflux, was added over a 2-hour period a solution of 4.4 g. (0.011 mole) of the dimethyl ester (XI) in 75 ml. of toluene and, after addition was complete, the mixture was heated under reflux for 11 hours longer. After cooling the reaction mixture, the excess potassium was cautiously decomposed with 5 ml. of methanol and then 50 ml. of water was added. The toluene layer was washed with water and dried over magnesium sulfate, and the toluene was evaporated under reduced pressure leaving crude 1,2,3,9-tetramethoxy-6-carbomethoxydibenzo[a,c][1,3]cycloheptadiene-7-one. This crude β-keto-ester was dissolved in 90 ml. of methanol, a solution of 5 g. of potassium hydroxide in 10 ml. of water was added, and the solution was allowed to stand at room temperature overnight followed by heating under reflux for one hour to ensure complete saponification of any ester. The basic solution was then acidified with 15 ml. of concentrated hydrochloric acid and heated under reflux overnight after which 100 ml. of water was added and the methanol evaporated with an air stream. The ketone was extracted with three 15-ml. portions of chloroform, and the chloroform extracts were washed with 30 ml. of water, 30 ml. of 1 *N* NaOH, and again with 30 ml. of water. After drying over magnesium sulfate, the chloroform was removed on the steam-bath and the residual ketone crys-

tallized from 20 ml. of methanol; yield 2.4 g. (69%). The analytical sample was further purified by a short path distillation at 170–180° (bath temperature) at 0.05 mm., m.p. 140.5–141°.

Anal. Calcd. for $C_{19}H_{20}O_5$: C, 69.5; H, 6.1. Found: C, 69.3; H, 6.2.

The semicarbazone of the ketone was prepared by heating it under reflux with semicarbazide hydrochloride and sodium acetate in aqueous methanol for 20 minutes and was crystallized from chloroform–ethanol, m.p. 246–246.5°.

Anal. Calcd. for $C_{20}H_{23}N_3O_5$: C, 62.3; H, 6.0; N, 10.9. Found: C, 62.6; H, 6.0; N, 10.3.

The oxime was obtained by heating under reflux for 15 hours a solution of 3.0 g. (9.2 millimoles) of the ketone, 3 g. of hydroxylamine hydrochloride and 3 ml. of pyridine in 30 ml. of ethanol. Evaporation of the solution to dryness on the steam-bath with an air stream left a residue which was digested with two 15-ml. portions of water. The remaining insoluble material was crystallized from methanol to give 2.4 g., 77% yield, of oxime, m.p. 194–196°.

Anal. Calcd. for $C_{19}H_{21}O_5N$: C, 66.5; H, 6.2; N, 4.1. Found: C, 66.5; H, 6.1; N, 4.0.

1,2,3,9-Tetramethoxydibenzo[a,c][1,3]cycloheptadiene (XIII).—A mixture of 0.5 g. (0.0015 mole) of the ketone, 0.35 g. of potassium hydroxide, 6.0 ml. of ethylene glycol, and 0.3 ml. of 85% hydrazine hydrate was heated according to the general procedure of Huang-Minlon.[32] After cooling, the reaction mixture was diluted with 8 ml. of water, acidified with concentrated hydrochloric acid, and extracted with three 10-ml. portions of benzene. The combined benzene extracts were washed with water, the benzene was evaporated, and the residue was shaken for 2 hours at room temperature with 35 ml. of 3 *N* NaOH and 5 ml. of dimethyl sulfate. This alkaline mixture was then extracted with three 10-ml. portions of benzene, and the residue after drying and evaporating the benzene was dissolved in 5 ml. of methanol, treated with carbon, and filtered hot. Cooling gave 0.29 g. (60%), m.p. 96–98°. A mixed melting point determination of this synthetic material with a sample of dihydrodeaminocolchinol methyl ether (m.p. 96–98°) obtained from the degradation of colchicine (below) showed no depression.

Anal. Calcd. for $C_{19}H_{22}O_4$: C, 72.6; H, 7.1. Found: C, 72.6; H, 7.0.

7-Amino-1,2,3,9-tetramethoxydibenzo[a,c][1,3]cycloheptadiene (II).—A solution of 1.36 g. (4 millimoles) of the oxime of XII in 30 ml. of 2 *N* ethanolic hydrochloric acid was hydrogenated at 30° and two atmospheres pressure using 50 mg. of platinum oxide and 100 mg. of 5% palladium-on-carbon as catalyst. After 48 hours, an additional 50 mg. of platinum oxide was added, and after another 48-hour period, hydrogenation ceased at 75% of the theoretical hydrogen absorption. The solution was filtered and evaporated to dryness and the residue was digested twice with 30-ml. portions of water. Crystallization of the insoluble material from methanol gave 0.32 g. of recovered oxime. The aqueous digests were made alkaline with potassium hydroxide and extracted with three 15-ml. portions of chloroform which were washed with 25 ml. of water, dried over magnesium sulfate, and evaporated to dryness. The crude solid amine (0.9 g.) was dissolved in 10 ml. of 1 *N* ethanolic hydrochloric acid and to this warm solution was added 20 ml. of ether. On cooling, 0.43 g., 39% yield based on unrecovered oxime, of the amine hydrochloride crystallized, m.p. 258–259°.

Anal. Calcd. for $C_{19}H_{24}ClNO_4$: C, 62.4; H, 6.6; Cl, 9.7. Found: C, 62.1; H, 6.6; Cl, 9.6.

The N-acetyl derivative of the amine was prepared by heating 100 mg. of the amine and 0.4 ml. of acetic anhydride on the steam-bath for 5 minutes after which 20 ml. of water was added and the heating continued for 30 minutes. Neutralization of the acetic acid with 1 *N* NaOH caused precipitation of a solid which was crystallized from 1 ml. of methanol and 1 ml. of water, m.p. 178–179°.

Anal. Calcd. for $C_{21}H_{25}NO_5$: C, 67.9; H, 6.8. Found: C, 67.7; H, 6.6.

Degradation of Colchicine.—Colchicine (I) was converted to colchicine by the method of Zeisel[33] and thence to N-acetyliodocolchinol following the directions of Windaus.[34] A 5% solution of the iodo compound in methanol containing three equivalents of sodium hydroxide was hydrogenated at room temperature and atmospheric pressure with 10% of palladium–carbon (5%) catalyst. The theoretical amount of hydrogen was absorbed, and a 90% yield of N-acetylcolchinol was obtained. This was treated in methanolic solution with ethereal diazomethane to prepare **N-acetylcolchinol methyl ether**, m.p. 201–202° (reported[35] m.p. 199°); $[\alpha]^{20}D$ −86.6° (*c*, 0.67, methanol).

Anal. Calcd. for $C_{21}H_{25}NO_5$: C, 67.9; H, 6.8. Found: C, 68.1; H, 6.9.

Colchinol methyl ether hydrochloride was prepared by adding 30 ml. of concentrated hydrochloric acid to a solution of 3 g. of N-acetylcolchinol methyl ether in 30 ml. of methanol and heating the mixture under reflux for 24 hours. The solution was then concentrated to dryness on the steam bath using an air stream, and the residue was suspended in water. After being made strongly alkaline with sodium hydroxide, the suspension was extracted with three 25-ml. portions of chloroform and the combined extracts were dried over magnesium sulfate and evaporated. To the residue dissolved in 20 ml. of absolute ethanol was added 5 ml. of 4.5 *N* ethanolic hydrochloric acid followed by 50 ml. of absolute ether. Cooling gave 2.5 g. of the crystalline hydrochloride which was recrystallized from absolute alcohol–ether (1:2), m.p. 258–259° (reported[35] m.p. 254°); $[\alpha]^{20}D$ −88.7° (*c*, 0.76, ethanol).

Anal. Calcd. for $C_{19}H_{24}ClNO_4$: C, 62.4; H, 6.6. Found: C, 62.1; H, 6.4.

Colchinol methyl ether *d*-acid tartrate was prepared by adding a solution of 1.62 g. (10.8 millimoles) of *d*-tartaric acid in 5 ml. of water to a warm suspension of 1.77 g. (5.4 millimoles) of colchinol methyl ether in 5 ml. of water and heating until solution was complete. The acid tartrate crystallized on cooling and was recrystallized from water, m.p. 182–184°; $[\alpha]^{20}D$ −62.2° (*c*, 1.2, water).

Anal. Calcd. for $C_{23}H_{29}NO_{10}$: C, 57.6; H, 6.1. Found: C, 57.3; H, 6.0.

N-Benzylidenecolchinol methyl ether was formed when a solution of 1.0 g. (3 millimoles) of colchinol methyl ether and 0.5 g. (4.7 millimoles) of benzaldehyde in 5 ml. of methanol was warmed on the steam-bath for 15 minutes. After standing at room temperature for one hour, the crystalline material was filtered and recrystallized from isopropyl alcohol, m.p. 145–146°; $[\alpha]^{25}D$ +23.3° (*c*, 0.73, dioxane).

Anal. Calcd. for $C_{28}H_{27}NO_4$: C, 74.8; H, 6.5. Found: C, 75.0; H, 6.3.

Dihydrodeaminocolchinol Methyl Ether.—The procedure of Cook, *et al.*,[36] was used to degrade N-acetylcolchinol methyl ether to deaminocolchinol methyl ether and to hydrogenate the latter to dihydrodeaminocolchinol methyl ether, m.p. 96–98° (reported[36] 96–97°).

Racemization of Colchinol Methyl Ether.—A solution of 0.60 g. (1.4 millimoles) of N-benzylidenecolchinol methyl ether in 5 ml. of a 35% methanol solution of benzyltrimethylammonium hydroxide was heated under reflux for 5 hours after which 12 ml. of 6 *N* HCl was added and the solution concentrated to 5 ml. The solution was diluted with water to 10 ml., then concentrated to 5 ml., and the process was repeated two more times. The final solution from which the hydrochloride had begun to crystallize, was basified with potassium hydroxide and extracted with three 10-ml. portions of chloroform, and the combined chloroform extracts were washed with water, dried over magnesium sulfate, and evaporated. To the warm solution of the residue in 4 ml. of 2.1 *N* ethanolic hydrochloric acid was added 8 ml. of ether and the amine hydrochloride crystallized on cooling. Recrystallization from ethanol–ether (1:2) gave 0.15 g. (29% yield) of material melting at 258–259°; $[\alpha]^{25}D$ 0.0 (*c*, 1.04, water).

Anal. Calcd. for $C_{19}H_{24}ClNO_4$: OCH_3, 33.9. Found: OCH_3, 33.5.

This ***dl*-colchinol methyl ether hydrochloride** gave no depression in a mixed melting point determination with

(32) Huang-Minlon, THIS JOURNAL, **68**, 2487 (1946).

(33) S. Zeisel, *Monatsh.*, **7**, 557 (1886).

(34) A. Windaus, *Sitzber. heidelberg. Akad. Wiss., Math.-naturw. Klasse*, A 18 abh (1914).

(35) A. Windaus, *ibid.*, A16, abh. (1919).

(36) J. W. Cook, W. Graham, A. Cohen, R. W. Lapsley and C. A. Lawrence, *J. Chem. Soc.*, 322 (1944).

synthetic 7-amino-1,2,3,9-tetramethoxydibenzo[a,c][1,3]-cycloheptadiene hydrochloride.

Racemic colchinol methyl ether was converted to the acetyl derivative in the same manner as used above for the synthetic amine. The **N-acetyl-*dl*-colchinol methyl ether** melted at 180–181° and this melting point was not depressed on mixing with 7-acetamino-1,2,3,9-tetramethoxydibenzo-[a,c][1,3]cycloheptadiene (m.p. 178–179°). It had no detectable rotation.

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Incorporation of Glycine Carbon into Yeast Lipides[1]

BY SIDNEY WEINHOUSE, RUTH H. MILLINGTON AND MURRAY STRASSMAN

Fatty acids isolated from yeast grown in the presence of methylene-C^{14}-labeled glycine had a high content of C^{14}, distributed equally among all carbons of the chain. Fatty acids isolated from yeast grown in the presence of carboxyl-labeled glycine had negligible activity. These results suggest that fatty acids are formed from glycine through the steps: serine, pyruvate and acetate. Glycine α-carbon activity was found also in the unsaponifiable matter, and the methyl carbons of choline.

During the course of a study of glycine metabolism in yeast, it was found that when this organism was grown on glucose as the sole carbon source, together with a small quantity of glycine labeled in the methylene carbon with C^{14}, the radioactivity of the cell lipides was of such magnitude as to indicate a substantial incorporation of glycine carbon therein.[2] With carboxyl labeled glycine, however, the incorporation was so low that it could safely be concluded that the two glycine carbons follow different pathways in the conversion to lipides. The pertinent data are summarized in Table I.

TABLE I

C^{14} ACTIVITIES OF LIPIDES FROM YEAST GROWN WITH METHYLENE- OR CARBOXYL-LABELED GLYCINE. VALUES ARE COUNTS/MINUTE/5 SQ. CM. DISH OF SUBSTANCES COUNTED AS SUCH

	Methylene-labeled			Carboxyl-labeled	
Expt. no.	1	2	3	1	2
Fatty acids	1650	1330	608[a]	12	5[2]
Unsaponifiable matter	2050	1190	1860	1	2
Phospholipide fatty acids	1980[b]		763	12	6
Trimethylamine chloroplatinate	1400		350[c]	..	..

[a] Three mM. of non-isotopic sodium acetate added in this experiment. [b] Phospholipides from 1 and 2 combined. [c] Hydrochloride.

Inasmuch as acetate is already well-established as a precursor for the carbon skeleton of the fatty acids and at least part of the sterol molecule,[3] it seemed likely that the glycine methylene carbon is first incorporated into acetate. Altman[4] has found glycine methylene carbon in the bone marrow fats of rabbits and has suggested that acetate was the precursor; Sprinson[5] and Elwyn and Sprinson[6] have shown that both the glycine methylene carbon and serine β-carbon can be utilized for acetate synthesis in rats; and Barker *et al.*,[7] have demonstrated the conversion of glycine α-carbon to methyl and carboxyl of acetate in *Diplococcus glycinophylum*. On the basis of previous studies a reasonable reaction pathway for conversion of the methylene carbon of glycine to fatty acids can be formulated in the 5 equations

(1) $^*CH_2NH_2COOH \longrightarrow H^*COOH$

(2) $^*CH_2NH_2COOH + H^*COOH \longrightarrow HO^*CH_2{}^*CHNHC_2COOH$

(3) $HO^*CH_2{}^*CHNH_2COOH \longrightarrow {}^*CH_3{}^*COCOOH$

(4) $^*CH_3{}^*COCOOH \longrightarrow {}^*CH_3{}^*COOH + CO_2$

(5) $^*CH_3{}^*COOH \longrightarrow {}^*CH_3{}^*CH_2 \ldots\ldots {}^*CH_2{}^*COOH$

Reaction 1, the conversion of glycine methylene carbon to formate was noted by Sakami[8] and Siekevitz and Greenberg[9] in intact rats and in liver slices. The coupling of formate and glycine to yield serine, reaction 2, was also observed by the same authors.[9,10] The appearance of glycine carboxyl carbon in the serine carboxyl of *Torulopsis* yeast[11] can also be cited in favor in reaction 2. The conversion of serine to pyruvate has been demonstrated to occur in a variety of microorganisms and in cell-free extracts of animal liver.[12] Steps 4 and 5 require no comment since they have been well-established both in animals and yeast.[13–15]

Any fatty acids formed by this mechanism would be expected to be labeled in all positions of the chain, though not necessarily equally in all carbon atoms. Sakami[9] and Siekevitz and Greenberg[8] found that the serine formed by rats from α-labeled glycine had a preponderance of activity in

(1) Work done under contract with the U. S. Atomic Energy Commission and aided by a grant from the American Cancer Society recommended by the Committee on Growth of the National Research Council. The labeled compounds used in this study were obtained from Tracerlab, Inc., on allocation by the U. S. Atomic Energy Commission. This work will be included as part of a thesis to be submitted by Murray Strassman to the Graduate School of Temple University in partial fulfillment of the requirements for the Ph.D. degree.

(2) The distribution of glycine carbon in other components of the yeast cell is being investigated and will be reported separately.

(3) Bloch, *Physiol. Rev.*, **27**, 574 (1947).

(4) Altman, *J. Biol. Chem.*, **177**, 985 (1949).

(5) Sprinson, *ibid.*, **178**, 529 (1949).

(6) Elwyn and Sprinson, *ibid.*, **184**, 465 (1950).

(7) Barker, Volcani and Cardon, *ibid.*, **173**, 803 (1948).

(8) Sakami, *ibid.*, **179**, 495 (1949).

(9) Siekevitz and Greenberg, *ibid.*, **180**, 845 (1949).

(10) Sakami, *ibid.*, **176**, 995 (1948).

(11) Ehrensvard, Sperber, Saluste, Reio and Stjernholm, *ibid.*, **169**, 759 (1947).

(12) Chargaff and Sprinson, *ibid.*, **151**, 273 (1943).

(13) Anker, *ibid.*, **176**, 1338 (1948).

(14) Weinhouse, Millington and Lewis, THIS JOURNAL, **70**, 3680 (1948).

(15) Pihl, Bloch and Anker, *J. Biol. Chem.*, **183**, 441 (1950).