Anal. Calcd. for $C_{10}H_{19}N_2O_4Cl$: N, 10.50; Cl, 13.32. Found: N, 10.74; Cl, 13.08.

N-*o*-Nitrophenylsulfenyl-L-prolyl-L-phenylalanine methyl ester was prepared (a) by interaction of *o*-nitrophenylsulfenyl-L-proline dicyclohexylammonium salt (4.5 g., 0.01 mole), 2.1 g. (0.01 mole) of L-phenylalanine methyl ester hydrochloride, and 2.2 g. of N,N'-dicyclohexylcarbodiimide in the manner described above for the preparation of VI (procedure b). Treatment with isopropyl ether and a few ml. of ethyl acetate followed by cooling in the refrigerator caused the sirupy product to crystallize. After recrystallization from isopropyl ether containing a small amount of ethyl acetate the yield was 3 g. (70%) and the m.p. 91–92°, $[\alpha]^{18}D$ −7.6° (*c* 5, dimethylformamide).

Anal. Calcd. for $C_{21}H_{23}N_3O_5S$: N, 9.78; S, 7.44. Found: N, 9.88; S, 7.33.

(b) *o*-Nitrophenylsulfenyl-L-proline in the form of its dicyclohexylammonium salt was coupled with L-phenylalanine methyl ester by the diphenylphosphoryl method in the manner described for the preparation of VI (procedure c). Upon working up the mixture as described above 80% of the pure product was obtained, m.p. 91–92°.

L-Prolyl-L-phenylalanine Methyl Ester Hydrochloride.—Addition of hydrogen chloride in ether to an ethereal solution of 2.15 g. (0.005 mole) of the corresponding nitrophenylsulfenyl derivative and scratching led to the crystallization of the required product. After recrystallization from acetone–ether the yield was 1.4 g. (93%), m.p. 159°, $[\alpha]^{18}D$ −41.9° (*c* 2.5, water); reported[44] m.p. 157–158°, $[\alpha]D$ −41° (in water); reported[45] m.p. 162.5–163.5°, $[\alpha]D$ −41.8° (water).

Anal. Calcd. for $C_{15}H_{21}N_2O_3Cl$: N, 8.95; Cl, 11.33. Found: N, 8.95; Cl, 11.16.

N^{α}-*o*-Nitrophenylsulfenyl-N^{ϵ}-carbobenzoxy-L-lysyl-L-alanine methyl ester was prepared by interaction of N^{α}-nitrophenylsulfenyl-N^{ϵ}-carbobenzoxy-L-lysine dicyclohexylammonium salt (6.15 g., 0.01 mole), 1.4 g. of L-alanine methyl ester hydrochloride (0.01 mole), and 2.2 g. of N,N'-dicyclohexylcarbodiimide in the manner described for the preparation of VI (procedure b). The sirupy product was treated with petroleum ether and left to stand in the refrigerator, whereupon it solidified. The material was suspended in warm diisopropyl ether, the necessary amount of warm ethyl acetate required to give a solution was added, and the solution was cooled in the refrigerator. The amorphous product was filtered off and was washed with a small amount of diisopropyl ether. The yield was 4.6 g. (88%), m.p. 74–77°, $[\alpha]^{20}D$ −36.6° (*c* 3, dioxane).

Anal. Calcd. for $C_{24}H_{30}N_4O_7S$: N, 10.79; S, 6.17. Found: N, 11.35; S, 5.76.

N-*o*-Nitrophenylsulfenyl-L-prolyl-N^{ϵ}-carbobenzoxy-L-lysyl-L-alanine Methyl Ester.—Ether containing hydrogen chloride was added to a suspension of 2.6 g. (0.005 mole) of N^{α}-nitrophenylsulfenyl-N^{ϵ}-carbobenzoxy-L-lysyl-L-alanine methyl ester in a mixture of ethyl acetate–ether (1:1). The solvent was decanted from the precipitated sirupy hydrochloride of the dipeptide ester, which was washed with decantation with ether several times. The residue, which after evaporation and drying weighed 1.88 g., was dissolved in chloroform together with 2.15 g. of *o*-nitrophenylsulfenyl-L-proline dicyclohexylammonium salt and 1.1 g. of N,N'-dicyclohexylcarbodiimide. The mixture was allowed to stand overnight at room temperature and was worked up as described for the preparation of VI (procedure b). The crude product was dissolved in tetrahydrofuran, the solution was filtered, and the filtrate was evaporated to dryness. The product crystallized upon the addition of petroleum ether and was recrystallized from methanol. The yield was 1.5 g. (50%), m.p. 175–176°, $[\alpha]^{20}D$ −44.2° (*c* 4, dimethylformamide).

Anal. Calcd. for $C_{29}H_{37}N_5O_8S$: N, 11.37; S, 5.20. Found: N, 11.32; S, 5.47.

L-Prolyl-N^{ϵ}-carbobenzoxy-L-lysyl-L-alanine Methyl Ester Hydrochloride.—Ether was added to a solution of 3.1 g. (0.005 mole) of the nitrophenylsulfenyl derivative of the tripeptide ester in 8 ml. of 1.4 *N* hydrogen chloride in methanol. The precipitated sirup was triturated with several portions of ether. Finally, it was suspended in warm ethyl acetate and the necessary amount of methanol required to give a solution was added. Upon addition of ether and cooling, the above ester hydrochloride crystallized. It was collected by filtration, washed with ether, and was kept in a desiccator over phosphorus pentoxide. The yield was 1.5 g. (60%), m.p. 133–135°, $[\alpha]^{20}D$ −60.6° (*c* 3.5, water).

Anal. Calcd. for $C_{23}H_{35}N_4O_6Cl$: N, 11.22; Cl, 7.11. Found: N, 11.01; Cl, 7.29.

S-Trityl-L-cysteinylglycine *p*-Nitrophenyl Ester Hydrochloride.—To a solution of 2.8 g. (0.01 mole) of glycine *p*-nitrophenyl ester hydrobromide, 5.2 g. of S-trityl-N-nitrophenylsulfenyl-L-cysteine, and 1.4 ml. of triethylamine in 30 ml. of chloroform, 2.2 g. of dicyclohexylcarbodiimide was added. The mixture was allowed to stand at room temperature overnight and was worked up as described for the preparation of VI (procedure a). The protected dipeptide ester thus obtained was a sirup, which was dissolved in ethyl acetate. Addition of ether containing hydrogen chloride to this solution gave the required dipeptide ester hydrochloride as an amorphous powder. The yield was 4 g. (68%), $[\alpha]^{20}D$ +39.6° (*c* 3, ethanol); reported[46] $[\alpha]D$ +39.6° (ethanol).

Anal. Calcd. for $C_{30}H_{28}N_3O_5SCl$: N, 7.27; S, 5.55; Cl, 6.13. Found: N, 7.23; S, 5.12; Cl, 6.28.

N-Tritylglycylglycine ethyl ester was prepared by interaction of Ia with glycine ethyl ester in the manner described above for the preparation of N-tritylsulfenyl-L-phenylalanylglycine ethyl ester. The crude product was recrystallized from ethanol. The yield was 70%, m.p. 161° (reported[47] 161°).

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[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY, UNIVERSITY OF MINNESOTA, MINNEAPOLIS 14, MINN.]

The Biosynthesis of the Alkaloids of *Colchicum*. III. The Incorporation of Phenylalanine-2-C^{14} into Colchicine and Demecolcine[1]

BY EDWARD LEETE[2]

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DL-Phenylalanine-3-C^{14} was fed to *Colchicum byzantinum* corms, alone and in the presence of relatively large amounts of inactive gallic, protocatechuic, and *p*-hydroxybenzoic acids. The two former compounds partially inhibited the incorporation of phenylalanine-3-C^{14} into colchicine and demecolcine. However, the alkaloids isolated from corms which had been fed gallic acid-carboxyl-C^{14} were devoid of radioactivity. When DL-phenylalanine-2-C^{14} was fed to *Colchicum* corms, radioactive alkaloids were obtained and systematic degradation established that they had more than 90% of their activity located in ring B at C_6.

It has been previously shown[3] that the administration of phenylalanine-3-C^{14} to *Colchicum byzantinum* corms led to the formation of radioactive colchicine (I) which had essentially all its activity located in ring B at C_5. However, the radioactive colchicine obtained when tyrosine-2-C^{14} was fed to *Colchicum autumnale* plants was not labeled at one specific position.[4] No activity was located at C_6 and considerable activity was detected in the N-acetyl group, indicating that the tyrosine-2-C^{14} had undergone metabolic breakdown in the plant, the resultant fragments being then incorporated into colchicine. These results led Battersby[5]

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(2) Alfred P. Sloan Fellow, 1962–1964.

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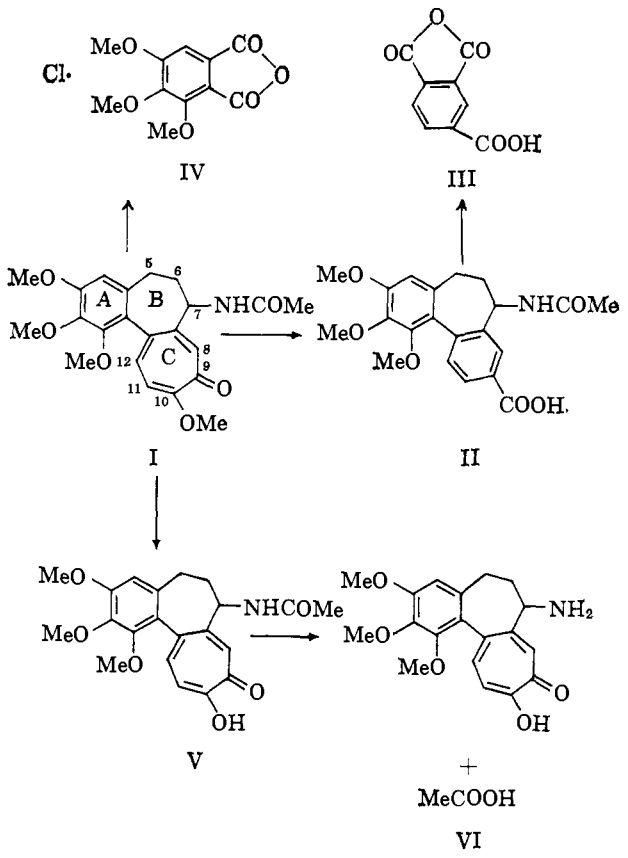

Fig. 1.—Degradation of the radioactive colchicine.

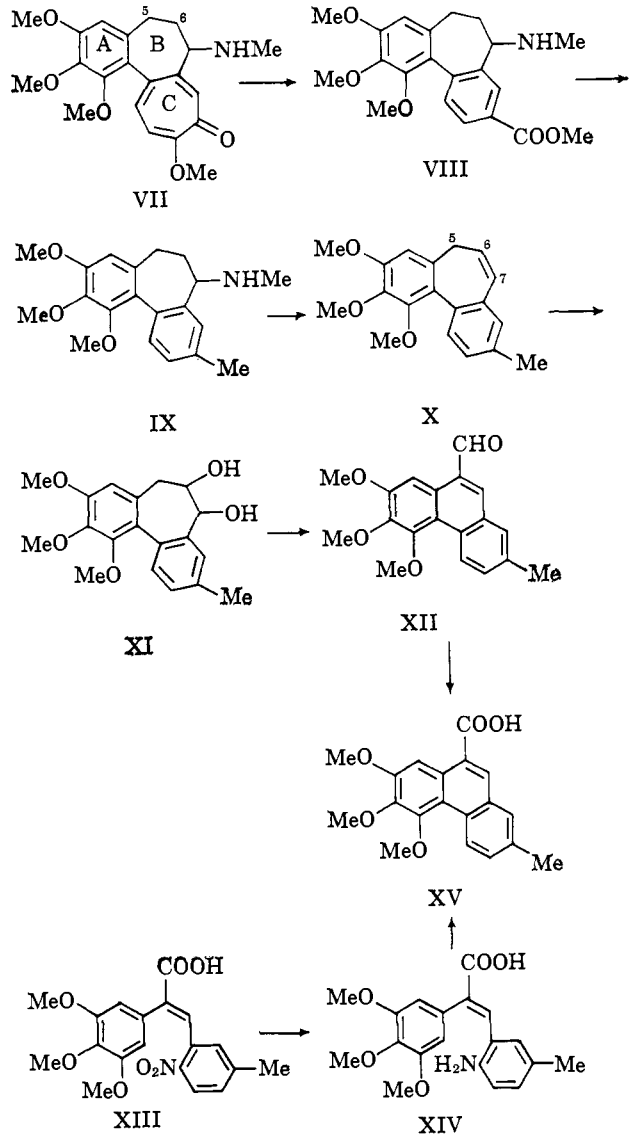

Fig. 2.—Degradation of the radioactive demecolcine.

to suggest that a C_6–C_1 unit, such as gallic acid, was involved in the biosynthesis of ring A and C_5 of ring B.

We therefore fed phenylalanine-3-C^{14} to *C. byzantinum* corms in the presence of relatively large amounts of nonradioactive gallic, protocatechuic, and *p*-hydroxybenzoic acids. It was considered that if any of these compounds were intermediates between phenylalanine and colchicine, a reduction in the incorporation of tracer into colchicine and the concomitant demecolcine (VII) would occur. Gallic and protocatechuic acid did significantly lower the incorporation of tracer into the alkaloids (*cf.* Table I, Experimental). However, when gallic acid-carboxyl-C^{14} was fed to *C. byzantinum* corms, the resultant alkaloids were completely inactive. Thus although gallic acid is apparently not a precursor of colchicine and democolcine, it does interfere with the enzyme systems responsible for the biosynthesis of these alkaloids. Since gallic and protocatechuic acid are readily oxidized, it seems plausible that they would reduce the availability of biological oxidizing agents which are obviously required for the hydroxylation of ring A and may be involved in the coupling of rings A and C.[6] On the other hand, *p*-hydroxybenzoic acid, which does not undergo ready autoxidation, had very little effect on the incorporation of phenylalanine-3-C^{14} into the alkaloids.

Concurrent with the above experiments we also fed phenylalanine-2-C^{14} to *C. byzantinum* corms and obtained radioactive colchicine and demecolcine, a significant incorporation (0.08 and 0.10%, respectively) of tracer having occurred. The colchicine was degraded as illustrated in Fig. 1. Colchinoic acid (allocolchiceine) (II) was obtained by the action of methanolic sodium methoxide on the alkaloid.[7] This acid was demethylated with hydrobromic acid and the resultant pyrogallol derivative oxidized with alkaline potassium permanganate to yield trimellitic anhydride (III). Oxidation of colchicine with alkaline potassium ferricyanide afforded 3,4,5-trimethoxyphthalic anhydride (IV). Hydrolysis with dilute acid yielded colchiceine (V), while stronger acid for a prolonged time afforded trimethylcolchicinic acid (VI)[8] and acetic acid, collected as the sodium salt. All the carbons of colchicine except C_6 and the O-methyl group at C_{10} are accounted for in the degradation products trimellitic anhydride, sodium acetate, and 3,4,5-trimethoxyphthalic anhydride. Since the combined specific activity of these compounds (*cf.* Table II) is less than 10% that of the colchicine, more than 90% of the radioactivity must be located at C_6, the O-methyl group at C_{10} having negligible activity. It was thus highly desirable to determine the activity at C_6 directly. Most of the radioactive colchicine had been used up in the previously described degradations. However, we had available the radioactive demecolcine derived from phenylalanine-2-C^{14}. It was considered highly improbable that colchicine and demecolcine would be produced by different biosynthetic reactions and we therefore expected the demecolcine to be also labeled mainly at C_6. The degradation, illustrated in Fig. 2, which we carried out on the demecolcine, is essentially

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the same as that used by Battersby and Reynolds[4] on colchicine derived from tyrosine-2-C^{14}. Treatment of the alkaloid with methanolic sodium methoxide yielded demecolchinoic acid, which was esterified with diazomethane affording the ester VIII. Reduction of this ester with lithium aluminum hydride followed by hydrogenolysis of the resultant benzyl alcohol with hydrogen in the presence of palladium-on-charcoal yielded the amine IX. This compound was subjected to exhaustive methylation affording trimethylamine, collected as tetramethylammonium iodide, and 1,2,3-trimethoxy-9-methyldibenzo[a,c]-$\Delta^{1,3,5}$-cycloheptatriene (X). The latter compound was also obtained by a similar series of reactions from colchicine. The 6,7-double bond was hydroxylated with osmium tetroxide yielding the diol XI which was cleaved with lead tetraacetate, the resultant dialdehyde cyclizing in faintly alkaline methanol to 2,3,4-trimethoxy-7-methylphenanthrene-10-aldehyde (XII). Oxidation of this aldehyde with potassium permanganate in acetone afforded the corresponding carboxylic acid XV. This acid was also prepared by a series of reactions analogous to those used by Barton, *et al.*,[9] for the synthesis of 2,3,4,7-tetramethoxyphenanthrene-10-carboxylic acid. A Perkin reaction involving 2-nitro-5-methylbenzaldehyde and 3,4,5-trimethoxyphenylacetic acid afforded 2-nitro-5-methyl-α-(3′,4′,5′-trimethoxyphenyl)-cinnamic acid (XIII). The amino acid XIV obtained by reduction of this nitro compound with ferrous sulfate and ammonia was converted to the phenanthrene carboxylic acid by the Pschorr reaction. The radioactive phenanthrene carboxylic acid derived from demecolcine was decarboxylated by heating in quinoline in the presence of copper chromite, the resultant carbon dioxide being collected as barium carbonate. It was found that this barium carbonate had the same specific activity as the demecolcine, indicating that the demecolcine was labeled solely at C_6.

These results, considered together with our earlier observations,[3] strongly suggest that demecolcine and colchicine are derived from phenylalanine by biosynthetic reactions in which at least two carbons of the side chain are retained. It is tempting to speculate that the carboxyl group of phenylalanine is also incorporated into the alkaloids, appearing at C_7. We are currently carrying out feeding experiments to test this hypothesis. Since Battersby and Reynolds found that tyrosine-2-C^{14} was not incorporated specifically into colchicine, we can deduce that phenylalanine is not converted into tyrosine prior to its incorporation into rings A and B of colchicine. A similar situation has been discovered in recent studies on the biosynthesis of the *Amaryllidaceae* alkaloids. It was found that ring A of lycorine which contains a methylenedioxy group was derived from phenylalanine, whereas labeled tyrosine was not incorporated into this part of the molecule.[10,11]

Experimental[12]

Assay of the Radioactive Compounds.—Some of the radioactive compounds were assayed on aluminum planchets in a Nuclear Chicago Model C-115 low background Q-gas flow counter, making corrections for self absorption and geometry. When possible, samples were counted in a Nuclear Chicago Model 724 liquid scintillation spectrometer using as solvents either (a) toluene containing 0.5% 2,5-diphenyloxazole (PPO) and 0.03% 1,4-bis-2-(5-phenyloxazolyl)benzene (POPOP); or (b) dioxane–water mixtures containing 10% naphthalene, 0.7% PPO, and 0.05% POPOP. Barium carbonate was counted with high precision by decomposing with concentrated sulfuric acid in a closed system, collecting the liberated carbon dioxide in methanolic Hyamine-10X solution[13] which was then diluted with the previously mentioned toluene solution containing the scintillators. Absolute counting efficiencies of 50–65% with a background of 22–30 c.p.m. were obtained with this liquid scintillation spectrometer. Activities were always determined in duplicate and agreements better than 5% were usually obtained.

Gallic Acid-Carboxyl-C^{14}.—3,4,5-Trimethoxybenzoic acid-carboxyl-C^{14} was prepared by the carboxylation of 3,4,5-trimethoxyphenyllithium using essentially the same procedure as that described in the literature.[14] This acid (140 mg.) was refluxed with 47% hydriodic acid (1.5 ml.) for 2 hr. in a nitrogen atmosphere. On cooling the reaction mixture, gallic acid monohydrate separated out and was filtered off, washed with ice-cold water, and dried *in vacuo*. The yield was 87.6 mg. (70%).

Administration of Tracers to *Colchicum byzantinum* Corms and Isolation of the Colchicine and Demecolcine.—Solutions of the tracers were fed to sprouting *C. byzantinum* corms by means of a cotton wick inserted into the outer layer of the corm as previously described.[3] All the feedings were carried out at the same time in October, 1962, in the brightly lit, temperature-controlled (22°), nicotine-free office of the author. Ten corms were used in each feeding experiment. In the experiments involving inhibitors, 30 mg. of each of these was fed to the corms 24 hr. before adding the phenylalanine-3-C^{14}. Distilled water was fed to the control plants. On the day that the tracer was fed and on the 9 following days 10 mg. of each of the inhibitors was fed to the corms *via* the cotton wicks. All the plants were watered each day with distilled water *via* the wicks. After 21 days the plants (average wt. 1100 g.) were harvested and macerated in a Waring blendor with methanol (3 l.). After standing between 1 and 7 days the slurry was filtered through cloth and the filtrate evaporated to 400 ml. in rotary evaporator. This solution was filtered through Celite and the filtrate extracted with petroleum ether (b.p. 60–70°; 3 × 100 ml.). Sodium bicarbonate (10 g.) was added to the aqueous layer which was extracted with chloroform (4 × 200 ml.). Evaporation of the dried chloroform extract yielded the crude alkaloids (average wt. 1.5 g.). A chromatographic column (25 × 4 cm.) was made with neutral Woelm alumina (activity III) (200 g.) using a slurry in benzene. The alkaloids dissolved in a 1:1 mixture of benzene and chloroform were added to the column which was then eluted with a 1:1 mixture of benzene and chloroform (1 l.). The column was then eluted with pure chloroform, 30-ml. fractions being taken. Demecolcine was usually detected in fractions 14 to 25. After fraction 30 the column was eluted with 10% ethanol in chloroform, colchicine being then found in fractions 32 to 36. Both alkaloids were crystallized from a mixture of ethyl acetate and ether. The average recovery of pure demecolcine and colchicine was 200 and 180 mg., respectively. Details of the amounts of tracers fed and the specific activities of the isolated alkaloids are recorded in Table I.

TABLE I

Compounds fed to *C. byzantinum*			Activity of the alkaloids, d.p.m./mmole	
Compound	Amount, mg.	Activity, d.p.m.	Colchicine	Demecolcine
DL-Phenylalanine-3-C^{14} (no additions)	3.38	1.28×10^8	8.1×10^4	10.0×10^4
DL-Phenylalanine-3-C^{14} and *p*-hydroxybenzoic acid	3.38 130	1.28×10^8	7.7×10^4 0	8.7×10^4
DL-Phenylalanine-3-C^{14} and protocatechuic acid	3.38 130	1.28×10^8	3.0×10^4 0	3.7×10^4
DL-Phenylalanine-3-C^{14} and gallic acid	3.38 130	1.28×10^8 0	2.6×10^4	1.9×10^4
Gallic acid-carboxyl-C^{14}	19.1	1.9×10^8	0	0
DL-Phenylalanine-2-C^{14}	36.8	2.2×10^8	3.3×10^5	4.1×10^5

Degradation of the Alkaloids Derived from Phenylalanine-2-C^{14}.—All the degradations carried out on the colchicine have been previously described and references to the procedures used are recorded in Table II. The demecolcine from the plant was diluted with an equal weight of inactive alkaloid and the degradation proceeded through the following compounds:

(a) **1,2,3-Trimethoxy-9-methyldibenzo[a,c]-$\Delta^{1,3,5}$-cycloheptatriene (X).**—Demecolcine (200 mg.) was added to a solution of sodium (200 mg.) in methanol (5 ml.) and the mixture refluxed for 1 hr. After standing for an additional hr., water (2 ml.) was added and the mixture evaporated to dryness *in vacuo*. The residue was dissolved in water and the solution neutralized with 2 *N* hydrochloric acid. The pale yellow solution was extracted with chloroform which was dried over sodium

(9) N. Barton, J. W. Cook, and J. D. Loudon, *J. Chem. Soc.*, 176 (1945).

(10) R. J. Suhadolnik, A. G. Fischer, and J. Zulalian, *J. Am. Chem. Soc.*, **84**, 4348 (1962).

(11) W. C. Wildman, A. R. Battersby, and S. W. Breuer, *ibid.*, **84**, 4599 (1962).

(12) Melting points are corrected. We thank Mrs. Olga Hamerston and her assistants at the University of Minnesota for the analyses.

(13) Registered trade mark of Rohm and Haas; *p*-(diisobutylcresoxyethoxyethyl)dimethylbenzylammonium hydroxide.

(14) P. Numerof, M. Gordon, and J. M. Kelly, *J. Pharmacol. Exptl. Therap.*, **115**, 427 (1955).

TABLE II

ACTIVITIES OF THE DEGRADATION PRODUCTS OF COLCHICINE AND DEMECOLCINE DERIVED FROM PHENYLALANINE-2-C^{14}

	Activity, d.p.m./mmole
Colchicine	3.3×10^5
Colchiceine[15]	3.3×10^5
Colchinoic acid[8]	3.3×10^5
Trimellitic anhydride[7]	0.04×10^5
Trimethylcolchicinic acid[15]	3.0×10^5
Sodium acetate[15]	0.14×10^5
3,4,5-Trimethoxyphthalic anhydride[3]	0.13×10^5
Demecolcine	4.1×10^5
Dibenzocycloheptatriene X	4.1×10^5
Tetramethylammonium iodide	0
Phenanthrenecarboxylic acid XV	4.2×10^5
Barium carbonate (from the decarboxylation of XV)	4.1×10^5

sulfate and evaporated yielding demecolchinoic acid (allodemecolceine).[16] This acid was added to an ether solution of diazomethane formed from 3 g. of nitrosomethylurea. After standing overnight, the ether was evaporated and the residue refluxed for 4 hr. in ether (100 ml.) containing lithium aluminum hydride (1 g.). Water (3 ml.) was then added and the mixture refluxed for 1 hr. The mixture was filtered and the residue washed with ether. Evaporation of the filtrate and washings yielded an oily residue which was dissolved in acetic acid (15 ml.) and hydrogenated for 3 hr. at 2 atm. pressure in the presence 10% palladium-on-charcoal catalyst (300 mg.). The reaction mixture was filtered, made alkaline by the addition of 10% sodium hydroxide solution, and extracted with ether (500 ml.). The ether extract was extracted with 2 *N* hydrochloric acid which was then made basic with sodium hydroxide and re-extracted with ether. The dried ether solution was evaporated to yield the amine IX (98 mg.). This amine was dissolved in methanol (10 ml.) and refluxed with methyl iodide (1 ml.) and sodium bicarbonate (200 mg.) for 18 hr. The mixture was then filtered and the filtrate on evaporation to small bulk afforded pale yellow prisms of 1,2,3-trimethoxy-9-methyl-7-dimethylamino-dibenzo[a,c]-$\Delta^{1,3}$-cycloheptadiene methiodide (103 mg.), m.p. 228–229°. This methiodide was dissolved in water (10 ml.) and stirred with silver hydroxide (from 120 mg. of silver nitrate) for 15 min. The mixture was filtered and the filtrate evaporated to dryness *in vacuo*, and the residue distilled at 220° (10^{-2} mm.) affording trimethylamine, which was condensed in a liquid nitrogen trap, and 1,2,3-trimethoxy-9-methyl-dibenzo[a,c]-$\Delta^{1,3,5}$-cycloheptatriene which was obtained as a colorless oil (51 mg.). Crystallization from aqueous methanol afforded colorless plates, m.p. 117–118°. This product was identical (mixture m.p., infrared spectrum) with material obtained from colchicine by a similar series of reactions which were outlined by Battersby and Reynolds in their preliminary communication.[4]

Anal. Calcd. for $C_{19}H_{20}O_3$: C, 77.00; H, 6.80. Found: C, 76.41; H, 6.78.

The trimethylamine in the liquid nitrogen trap was washed out with ethanol. Methyl iodide (1 ml.) was added to this solution which was then evaporated after standing overnight, yielding tetramethylammonium iodide.

(b) **6,7-Dihydroxy-1,2,3-trimethoxy-9-methyl-dibenzo-[a,c]-$\Delta^{1,3}$-cycloheptadiene (XI).**—The cycloheptatriene derivative X (99 mg.) was dissolved in ether (5 ml.) and osmium tetroxide (93 mg.) added followed by a drop of pyridine. After standing for 2 hr. at room temperature the mixture was filtered and the buff colored residue was dissolved in methanol (20 ml.), mixed with a solution of sodium sulfite (0.9 g.) in water (10 ml.), and refluxed for 2 hr. The mixture was then filtered and the residue washed with hot methanol. The combined filtrates were evaporated to dryness, the residue dissolved in water and extracted with ether. The dried ether extract was evaporated and the residue crystallized from aqueous methanol, affording colorless prismatic needles of the *cis*-6,7-diol (68 mg.), m.p. 189–190°.

Anal. Calcd. for $C_{19}H_{22}O_5$: C, 69.07; H, 6.71. Found: C, 68.59; H, 6.82.

(c) **2,3,4-Trimethoxy-7-methylphenanthrene 10-aldehyde (XII).**—The sublimed *cis*-diol XI (52.6 mg.) was dissolved in dry benzene (12 ml.) and lead tetraacetate (72 mg.) added to the boiling solution. After refluxing for 1 hr. the mixture was filtered and the filtrate washed with water and dried over sodium sulfate. The solution was evaporated and the residue crystallized from methanol containing a trace of sodium hydroxide affording pale yellow prismatic needles of the phenanthrene aldehyde (32.8 mg.), m.p. 94–95°.

Anal. Calcd. for $C_{19}H_{18}O_4$: C, 73.53; H, 5.85. Found: C, 73.31; H, 6.13.

(d) **2,3,4-Trimethoxy-7-methylphenanthrene-10-carboxylic Acid (XV).**—The phenanthrene aldehyde XII (32 mg.) and potassium permanganate (14 mg.) were dissolved in acetone (3 ml.) and allowed to stand at room temperature for 10 hr. Water was then added, followed by a little sodium sulfite and hydrochloric acid. The mixture was extracted with ether. The ether layer was extracted with 10% sodium hydroxide which was then acidified with hydrochloric acid and again extracted with ether. The final ether extract was dried over sodium sulfate, evaporated, and the residue sublimed (210°, 10^{-2} mm.), affording a pale yellow sublimate of the phenanthrenecarboxylic acid (16.5 mg.), m.p. 195–196°, having an infrared spectrum identical with the acid prepared by the unambiguous method described later.

(e) **Decarboxylation of the Phenanthrenecarboxylic Acid.**—The acid (19.6 mg.) was refluxed with copper chromite (20 mg.) in quinoline (2 ml.) in a current of nitrogen for 1 hr., the exit gases being passed through barium hydroxide solution yielding barium carbonate (7.5 mg., 63% yield).

2-Nitro-5-methyl-α-[3′,4′,5′-trimethoxyphenyl] cinnamic Acid (XIII).—2-Nitro-5-methylbenzaldehyde,[17] m.p. 39–40°, was obtained from the commercially available 2-nitro-5-methylbenzoic acid[18] *via* the acid chloride and the Reissert compound. The aldehyde (4.0 g.) was refluxed in acetic anhydride (20 ml.) with the finely powdered dry sodium salt of 3,4,5-trimethoxyphenylacetic acid (6.0 g.) in a nitrogen atmosphere for 10 hr. The mixture was then cooled, water added, and then refluxed for 1 hr. The mixture was then extracted with ether which was then washed several times with water. The dark brown ether solution was then extracted several times with 10% sodium carbonate solution. This solution was acidified with hydrochloric acid and the mixture extracted with ether. The residue obtained on evaporation of the dried ether solution was crystallized from benzene affording colorless plates (3.1 g.), m.p. 148–149°.

Anal. Calcd. for $C_{19}H_{19}O_7N$: C, 61.12; H, 5.13; N, 3.75. Found: C, 61.20; H, 5.22; N, 3.75.

2-Amino-5-methyl-α-[3′,4′,5′-trimethoxyphenyl] cinnamic Acid (XIV).—The nitro compound XIII (2.0 g.) was dissolved in water (32 ml.) and 12 *N* ammonia solution (8 ml.), and added to a stirred mixture of ferrous sulfate (16 g.) in water (40 ml.) and 12 *N* ammonia (40 ml.). The mixture was stirred at 70–80° for 1 hr. and then filtered. The pale yellow filtrate was brought to a pH of 5 with dilute hydrochloric acid when the amino acid crystallized out (1.8 g.). Recrystallization from ethanol, in which the amino acid dissolved with a bright yellow color, afforded colorless plates, m.p. 219–220° with decomposition, some softening occurring before this temperature.

Anal. Calcd. for $C_{19}H_{21}O_5N$: C, 66.46; H, 6.16; N, 4.08. Found: C, 65.83; H, 5.85; N, 3.91.

2,3,4-Trimethoxy-7-methylphenanthrene-10-carboxylic Acid (XV).—The amino acid XIV (480 mg.) was dissolved in 2 *N* sulfuric acid (6 ml.) and acetic acid (2 ml.) and cooled to 0°. Sodium nitrite (100 mg.) dissolved in water (2 ml.) was added and the mixture became bright yellow. Water (10 ml.) was added followed by solid sodium carbonate until the mixture became neutral. The solution was then warmed to 60° and made definitely alkaline with sodium hydroxide. After 2 hr. at this temperature hydrochloric acid was added producing a gelatinous precipitate. This precipitate was dried and then sublimed, the sublimate being crystallized from methanol when the phenanthrenecarboxylic acid was obtained as colorless prisms (105 mg.), m.p. 197–198°.

Anal. Calcd. for $C_{19}H_{18}O_5$: C, 69.92; H, 5.56. Found: C, 69.66; H, 5.47.

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(18) Purchased from K and K Laboratories, Inc., New York, N. Y.