crystalline cyclobuxoxine (329 mg.). Recrystallization from methanol–ether gave colorless plates: m.p. 181–183°; $[\alpha]^{27}D$ +169° (*c* 0.63, chloroform); λ_{max}^{Nujol} 2.82, 2.97, 5.91, 6.18, 11.18 μ; mass spectral peaks *m/e* 353 (M − 18), 338 (M − 18 − 15), 310 (M − 18 − 15 − 28).

Anal. Calcd. for $C_{24}H_{37}NO_2 \cdot 0.5CH_3OH$: C, 75.92; H, 10.14; N, 3.61. Found: C, 75.85; H, 10.28; N, 3.64.

O,N-Diacetylcyclobuxoxine (VII, $R^1 = R^2 = COCH_3$; R^3 = O).—A solution of cyclobuxoxine (20 mg.) in dry pyridine (2 ml.) and acetic anhydride (1 ml.) was allowed to stand at room temperature for 24 hr. Dilution with water (20 ml.) gave, after 4 hr. of standing, needles (19.5 mg.): m.p. 211–213°; $[\alpha]^{30}D$ +92° (*c* 0.25, chloroform); $\lambda_{max}^{CHCl_3}$ 5.74, 6.08, 8.05 μ.

Anal. Calcd. for $C_{28}H_{41}NO_4$: C, 73.81; H, 9.07; N, 3.07. Found: C, 73.55; H, 9.30; N, 3.21.

Dihydrocyclobuxoxine-a (VIII, R = H).—A solution of cyclobuxoxine (214 mg.) in 10% acetic acid in ethanol (20 ml.) was added to reduced platinum oxide (150 mg.) in the same solvent mixture (20 ml.). Hydrogenation was carried out at atmospheric pressure and room temperature for 3 hr. Filtration, evaporation of the solvent under reduced pressure, neutralization with 2 *N* ammonium hydroxide solution, extraction with chloroform, drying, and evaporation under reduced pressure yielded 210 mg. of colorless solid residue. Crystallization from acetone gave colorless needles (114 mg.), m.p. 198–200°, $[\alpha]^{30}D$ +100° (*c* 0.38, chloroform). Second and third crops totaled 81 mg.

Anal. Calcd. for $C_{24}H_{39}NO_2$: C, 77.16; H, 10.52; N, 3.75. Found: C, 77.01; H, 10.49; N, 3.87.

Dihydrocyclobuxoxine-b (VII, $R^1 = R^2 = H$; R^3 = ).—A solution of sodium borohydride (20 mg.) in methanol (2 ml.) was added dropwise at room temperature to a stirred solution of cyclobuxoxine (25 mg.) in methanol (2 ml.). The reaction mixture was refluxed on a steam bath for 2 hr. Treatment with 5% aqueous sodium bicarbonate, followed by extraction with chloroform, drying, and evaporation under reduced pressure yielded 19.5 mg. of colorless solid. Thin layer chromatographic analysis on silica gel G, using the upper layer of the system acetic acid–1-butanol–water (1:4:5) and spraying with Dragendorf reagent, showed a predominance of over 80% of the major product. Crystallization from methanol–ether gave colorless needles (10 mg.), m.p. 192–194°, $[\alpha]^{30}D$ +60° (*c* 0.05, chloroform).

Anal. Calcd. for $C_{24}H_{39}NO_2$: C, 77.16; H, 10.52; N, 3.75. Found: C, 76.90; H, 10.72; N, 3.65.

N-Chlorodihydrocyclobuxoxine-a (VIII, R = Cl).—A solution of dihydrocyclobuxoxine-a (50 mg.) in methylene chloride (2 ml.) was cooled to 0° and treated dropwise with stirring with a solution of N-chlorosuccinimide (20 mg.) in chloroform (1 ml.). After stirring for 10 min. at 0°, the solution was washed with water, dried, and evaporated to dryness under reduced pressure. A crystalline residue was obtained (55 mg.), m.p. 232° dec.

Ruschig Degradation of N-Chlorodihydrocyclobuxoxine-a.—The chloramine VIII (R = Cl, 55 mg.) was treated with a solution of sodium methoxide (100 mg.) in methanol (3 ml.), and the mixture was refluxed for 2 hr. After evaporation to dryness, water (5 ml.) was added. Extraction with chloroform, with subsequent drying and evaporation under reduced pressure, yielded a yellowish oil. The oil was dissolved in ethanol (6 ml.) and 6 *N* sulfuric acid (3 ml.), and the solution was allowed to stand at room temperature for 6 hr. The mixture was diluted with water and extracted with chloroform, and the chloroform extract was evaporated to dryness. The residue was filtered on Woelm neutral alumina, grade I (3 g.), using 5% ether in benzene (150 ml.). The crystalline residue (one–enone IX, 17.3 mg.) showed an infrared spectrum containing bands for a carbonyl group at 5.87 μ and for an α,β-unsaturated carbonyl group at 6.00 and 6.16 μ. A solution of the one–enone (17.3 mg.) in 10% acetic acid and ethanol (5 ml.) was added to 10% palladium on carbon (35 mg.) in the same solvent mixture (5 ml.). The hydrogen consumption under atmospheric pressure and at room temperature was complete in 30 min. Evaporation of the solvent under reduced pressure, followed by the addition of water (25 ml.), gave a crystalline solid, which was filtered and dried (16.8 mg.). Recrystallization from acetone–Skellysolve B gave colorless needles, m.p. 185–187°. The infrared spectrum was superimposable upon that of an authentic sample of 4,14α-dimethyl-9β,19-cyclo-5α-pregnane-3,20-dione (X) and the melting point was not depressed upon admixture with the authentic sample.

Tumor Inhibitors. IX.[1] Synthesis of 10-Nitro-1-phenanthroic Acid[2,3]

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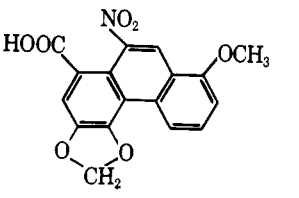
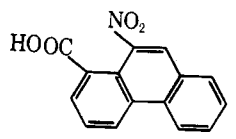
Received June 11, 1965

As part of a program on the synthesis of compounds structurally related to the naturally occurring tumor inhibitor, aristolochic acid (I),[5] we undertook the preparation of an analog with no oxygen–ether functions, namely, 10-nitro-1-phenanthroic acid (II). There were two principal methods available for the introduc-



I



II

tion of a nitro group into the phenanthrene nucleus.[6] The first involved direct nitration; the reaction affords a variety of isomers and is generally unsuited for the synthesis of specifically substituted compounds.[7] The second approach involved substitution of nitro for amino group in a Sandmeyer-type reaction. The latter method has been utilized effectively only for substituted nitrophenanthrenes and the yields for the majority of isomers seldom exceeded 10%.[8] Nevertheless, this method (Scheme I) was chosen in preference to the direct nitration method because of its specificity.

A Perkin condensation between the sodium salt of 2-bromophenylacetic acid (III)[9] and *o*-nitrobenzaldehyde (IV) afforded 2-bromo-2′-nitro-*cis*-stilbene-α-carboxylic acid (V). The nitro group was reduced using an ammoniacal solution of ferrous sulfate; a 65% yield of amino acid VI was obtained. 1-Bromo-10-phenanthroic acid (VIII) was then prepared by Pschorr cyclization of diazonium chloride VII. The method of Rutherford and Newman served to convert the acid into amine Xa *via* a variant of the Schmidt reaction.[10] A lithium–halogen exchange reaction, followed by a

(1) Part VIII: S. M. Kupchan, J. R. Knox, and M. S. Udayamurthy, *J. Pharm. Sci.*, **54**, 929 (1965).

(2) This investigation was supported in part by research grants from the National Cancer Institute (CA-04500) and the American Cancer Society (T-275).

(3) Abstracted from a part of the dissertation submitted by H. C. Wormser to the University of Wisconsin Graduate School, June 1965, in partial fulfillment of the requirements of the Ph.D. degree.

(4) American Foundation for Pharmaceutical Education Fellow, 1961–1963; National Institutes of Health Predoctoral Fellow, 1963–1965.

(5) S. M. Kupchan and R. W. Doskotch, *J. Med. Pharm. Chem.*, **5**, 657 (1962).

(6) A new approach to nitrophenanthrenes has subsequently been described: see S. M. Kupchan and H. C. Wormser, *Tetrahedron Letters*, 359 (1965); *J. Org. Chem.*, **30**, 3792 (1965).

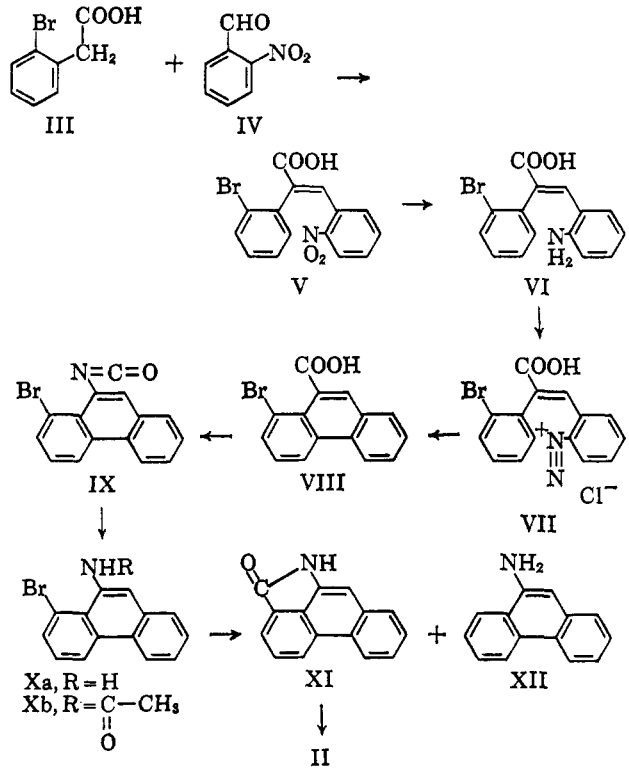
(7) M. J. S. Dewar and E. W. T. Warford, *J. Chem. Soc.*, 3570 (1956).

(8) P. M. G. Bavin and M. J. S. Dewar, *ibid.*, 4477 (1955).

(9) G. S. Misra and J. S. Shukla, *J. Indian Chem. Soc.*, **28**, 480 (1951).

(10) K. G. Rutherford and M. S. Newman, *J. Am. Chem. Soc.*, **79**, 213 (1957).

SCHEME I



carbonation step, afforded the lactam of 10-amino-1-phenanthroic acid (XI) along with the by-product XII, 9-aminophenanthrene. An attempt was made to synthesize XI from N-acetyl-1-bromo-10-phenanthrylamine (Xb) in the hope of improving the yield of the reaction, but this was unsuccessful. The lactam XI was hydrolyzed in a 5% sodium hydroxide in water–dioxane mixture, and the product was diazotized. Treatment of the diazonium solution with a large excess of sodium nitrite and copper salts[11] gave 10-nitro-1-phenanthroic acid (II), in low yield. The product showed an infrared spectrum (KBr) with bands at 6.60 and 7.45 μ (nitro group), and an ultraviolet spectrum [λ_{max}^{EtOH} 256.5 mμ (ϵ 37,200)] in good accord with that expected for structure II.[6] The compound was further characterized by direct comparison with a sample prepared by an alternate route.[6]

Experimental Section[12]

2-Bromo-2′-nitro-*cis*-stilbene-α-carboxylic Acid (V).—In a 250-ml., three-necked, round-bottom flask, equipped with a mechanical stirrer, water condenser, and thermometer were placed 2-bromophenylacetic acid[9] (III, as the sodium salt, 23.0 g., 0.0960 mole), *o*-nitrobenzaldehyde (IV, 23.0 g., 0.142 mole, m.p. 40°), and acetic anhydride (100 ml.). The reaction mixture was stirred and maintained at 100° for 48 hr. The excess acetic anhydride was then destroyed by the addition of 140 ml. of hot water, and, after boiling the reaction mixture for a few minutes, it was allowed to cool. The crude product was filtered and dried (26.4 g., m.p. 170–174°). Recrystallization from chloroform–Skellysolve B afforded an almost colorless, crystalline material (25.0 g., 75%): m.p. 195–197°; λ_{max} 5.91, 6.55, 7.45 μ; λ_{max}^{EtOH} 303 mμ (ϵ 4000) (plateau).

Anal. Calcd. for $C_{15}H_{10}BrNO_4$: C, 51.75; H, 2.90; N, 4.02. Found: C, 51.75; H, 2.97; N, 4.02.

2-Bromo-2′-amino-*cis*-stilbene-α-carboxylic Acid (VI).—A suspension of ferrous hydroxide was prepared by adding concentrated ammonium hydroxide solution (180 ml.) to a well-stirred solution of ferrous sulfate heptahydrate (72.0 g., 0.260 mole) in water (200 ml.). This rust-colored suspension was heated to 90°, and, while stirring, 2-bromo-2′-nitro-*cis*-stilbene-α-carboxylic acid (V, 12.0 g., 0.0320 mole, m.p. 195–197°) dissolved in ammonium hydroxide solution (1:1, 200 ml.) was added in small portions. After the addition was completed, the reaction was heated at 95° for an additional 15 min. The reaction mixture was filtered while hot and the residual black ferric hydroxide washed with an additional 100 ml. of hot water. The combined pale yellow filtrates were cooled and acidified to congo red with concentrated hydrochloric acid. The tan amino acid was collected and dried. The product was crystallized from absolute ethanol, yielding pale yellow microcrystals (7.13 g., 65%): m.p. 227–228°; λ_{max}^{EtOH} 284 mμ (ϵ 8850), 350 mμ (ϵ 5400). The infrared spectrum (Nujol mull) showed the disappearance of the 6.55- and 7.45-μ peaks (nitro).

Anal. Calcd. for $C_{15}H_{12}BrNO_2$: C, 56.62; H, 3.80; N, 4.40. Found: C, 56.77; H, 3.95; N, 4.45.

1-Bromo-10-phenanthroic Acid (VIII).—In a three-necked, 500-ml., round-bottom flask, equipped with a mechanical stirrer, water condenser with drying tube, and dropping funnel was added 2-bromo-2′-amino-*cis*-stilbene-α-carboxylic acid (VI, 6.0 g., 0.018 mole, m.p. 227–228°) in 100 ml. of hot 15% ethanolic hydrogen chloride solution. This yellow solution was cooled while stirring in a salt and ice bath until the internal temperature was −2°. A finely divided white precipitate of the hydrochloride of VI formed. Freshly prepared *n*-amyl nitrite (4 ml.) was then added dropwise during a period of 15 min. while maintaining the reaction temperature between 0 and −2°. Evidence of diazonium salt formation was obtained by means of an alkaline β-naphthol test solution (bright orange color spot test). Cold anhydrous ether (350 ml.) was added to the reaction mixture and the precipitated yellow diazonium salt (VII, 6.51 g., m.p. 114–116° with vigorous evolution of nitrogen) was filtered and dried. The infrared spectrum of this material (KBr) showed a strong and sharp peak at 4.40 μ (diazo group) and a peak at 5.87 μ (carboxyl). The dry diazonium chloride was suspended in dry acetone (50 ml.) and the Pschorr phenanthrene cyclization reaction was induced by the addition of Gatterman copper (150 mg.). A vigorous evolution of nitrogen occurred and the resulting clear orange acetone solution was filtered and poured into water (200 ml.). The white product was filtered, washed with water, and dried (4.84 g., m.p. 214–216°). Recrystallization from aqueous ethanol yielded colorless prisms (3.48 g., 61%), m.p. 215–217°, λ_{max}^{Nujol} 5.98 μ.

The methyl ester of VIII, which was prepared in quantitative yield by diazomethane methylation of the free acid, showed m.p. 69–70°; $\lambda_{max}^{CHCl_3}$ 5.81 μ; λ_{max}^{EtOH} 259.5 mμ (ϵ 51,800), 294 (11,400), 306 (11,850).

Anal. Calcd. for $C_{16}H_{11}BrO_2$: C, 60.97; H, 3.52. Found: C, 61.10; H, 3.55.

1-Bromo-10-phenanthryl Isocyanate (IX).—A solution of 1-bromo-10-phenanthroic acid (VIII, 1.0 g., 0.0033 mole, m.p. 215–217°) in hot glacial acetic acid (10 ml.) was added slowly to a cooled mixture of trifluoroacetic acid (10 ml.) and trifluoroacetic anhydride (10 ml.). The bright yellow solution was cooled in ice to 0° and sodium azide (0.500 g., 0.080 mole) was added in small portions while swirling the reaction mixture. The flocculent, crystalline, colorless precipitate which formed was filtered, washed with cold water, and dried (0.864 g., 85%): m.p. 142–145°, λ_{max}^{Nujol} 4.40 μ (isocyanate).

1-Bromo-10-phenanthrylamine (Xa).—To a 10-ml. flask equipped with a water condenser were added IX (0.500 g., 0.00164 mole, m.p. 142–145°), dioxane (3 ml.), and concentrated hydrochloric acid (2 ml.). The mixture was allowed to reflux for 1 hr. on a steam bath. Water (2 ml.) was added and the mixture was cooled in ice. The colorless crystalline product was

(11) H. H. Hodgson, A. P. Mahadevan, and E. R. Ward, *J. Chem. Soc.*, 1392 (1947).

(12) Melting points were determined on a Fisher-Johns melting point stage which had been calibrated with standard samples. Ultraviolet absorption spectra were determined in 95% ethanol on a Beckman (Model DK2A) recording spectrophotometer. Infrared absorption spectra were recorded in chloroform (unless otherwise specified) on a Beckman (Model 5A) double-beam infrared recording spectrophotometer. Microanalyses were by J. F. Alicino, Metuchen, N. J. Skellysolve B refers to petroleum ether, fraction boiling at 60–80°.

filtered and dried. This product was then thoroughly washed with ether and dried again (0.390 g., m.p. ~200°).

The hydrochloride salt was treated with 10% ammonium hydroxide solution (25 ml.) and the resulting base was collected and dried (0.374 g., 85%), m.p. 126–128°. The amine was recrystallized twice from aqueous ethanol to afford an analytical sample: m.p. 126–128°; $\lambda_{max}^{CHCl_3}$ 2.87, 2.95, 6.26, 6.72 μ; λ_{max}^{EtOH} 258 mμ (ϵ 33,800), 333 mμ (ϵ 8100).

Anal. Calcd. for $C_{14}H_{10}BrN$: C, 61.79; H, 3.71; N, 5.15. Found: C, 61.66; H, 3.92; N, 5.24.

Lactam of 10-Amino-1-phenanthroic Acid (XI).—In a 20-ml. three-necked, round-bottom flask, fitted with a water condenser with gas inlet tube, a rubber stopper injection port, and a Teflon stirring bar, was placed a solution of 1-bromo-10-phenanthrylamine (Xa, 0.100 g., 0.00034 mole, m.p. 126–128°) in anhydrous ether (3 ml.). The system was flushed with nitrogen and kept under this atmosphere throughout the reaction. Freshly prepared *n*-butyllithium solution (0.5 ml.) (made according to the method of Gilman, *et al.*[13]) was added dropwise to the stirred mixture by means of a hypodermic syringe. After 15 min., the mixture was carbonated by pouring it over powdered Dry Ice. The lithium salts were then decomposed by the addition of 10% hydrochloric acid (15 ml.) and the product was extracted with ether. The ethereal extract was washed with water and dried over sodium sulfate. Evaporation of the ether afforded an oily brown product (0.081 g.) which was chromatographed on acid-washed alumina. Chloroform–Skellysolve B (1:9) eluted a colorless crystalline compound (0.023 g.): m.p. 139–140°; $\lambda_{max}^{CHCl_3}$ 2.92, 3.0 (N–H stretching), 6.27, 6.67 μ (aromatic). This compound gave a negative halogen test on sodium fusion. The ultraviolet spectrum of this product was found to be superimposable with that of 9-aminophenanthrene (lit.[14] m.p. 137–138°).

Chloroform–Skellysolve B (1:3) eluent yielded the desired lactam as a strongly fluorescent solid which was recrystallized from chloroform–Skellysolve B (12 mg., m.p. 235°) (15% yield): λ_{max}^{EtOH} 221.5 mμ (ϵ 51,000) 230 (28,000), 244 (27,500), 284 (21,000), 295 (22,000); λ_{max}^{KBr} 3.10 (N–H stretching), 6.00 μ (lactam carbonyl).

Anal. Calcd. for $C_{15}H_9NO$: C, 82.17; H, 4.14; N, 6.39. Found: C, 81.95; H, 4.33; N, 6.36.

N-Acetyl-1-bromo-10-phenanthrylamine (Xb).—Acetylation of Xa (50 mg., 0.00018 mole, m.p. 126–128°) with a tenfold excess of acetic anhydride yielded, after work-up, shiny colorless crystals (42 mg., 73%), m.p. 208–209.5°. Recrystallization from aqueous ethanol gave an analytical sample: m.p. 210–210.5°; λ_{max} 2.91 (N–H stretching), 5.95 μ (amide carbonyl).

Anal. Calcd. for $C_{16}H_{12}BrNO$: C, 61.16; H, 3.85; N, 4.46. Found: C, 61.01; H, 3.85; N, 4.57.

10-Nitro-1-phenanthroic Acid (II).—A suspension of the lactam XI (0.022 g., 0.0001 mole, m.p. 235°) in 5% sodium hydroxide solution (2 ml.) was stirred and refluxed in an oil bath overnight. The yellow solution was cooled and sodium nitrite (0.069 g., 0.0010 mole) was added. This solution was added dropwise to 5% hydrochloric acid (10 ml.) cooled to 0°. A positive alkaline β-naphthol test was obtained. The diazonium solution was then added dropwise to a mixture of sodium nitrite (4.50 g.), copper sulfate pentahydrate (0.75 g.), sodium bicarbonate (3.5 g.), and cuprous oxide (0.5 g.) in water (100 ml.). The pH of the mixture remained at about 7.5 throughout a 12-hr. period. The mixture was filtered and the filtrate was acidified with hydrochloric acid to congo red. The small precipitate which had formed was filtered and dried (5 mg.), m.p. 240–246°. This product was recrystallized once from glacial acetic acid and once from ethanol to afford tan needles (2 mg.): m.p. 268–270°; λ_{max}^{EtOH} 256 mμ (ϵ 37,200); λ_{max}^{KBr} 6.60, 7.45 μ (nitro).

Anal. Calcd. for $C_{15}H_9NO_4$: C, 67.41; H, 3.39; N, 5.24. Found: C, 67.57; H, 3.62; N, 5.34.

The melting point was undepressed by admixture with a sample (m.p. 268–270°) prepared by an alternate route[6] and the infrared spectrum (KBr) was superimposable upon that of the comparison sample.

(13) H. Gilman, J. A. Beel, C. G. Brannen, M. W. Bullock, G. E. Dunn, and L. S. Miller, *J. Am. Chem. Soc.*, **71**, 1499 (1949).

(14) M. A. Goldberg, E. P. Ordas, and G. Carsch, *ibid.*, **69**, 260 (1947).

Tumor Inhibitors. XI.[1] Proof of Structure of Aristolochic Acid-C by Total Synthesis of Its Methyl Ester Methyl Ether[2,3]

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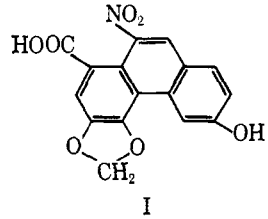
Received June 11, 1965

Following the discovery of a water-soluble quaternary base, magnafluorine, in the basic component of the Chinese drug "Fang Chi," the acid and neutral components were examined by Tomita and co-workers.[5] The acid fraction yielded aristolochic acid-I[1] and two nitrophenanthroic acids, aristolochic acid-B and aristolochic acid-C. Crystalline neutral substances isolated were aristololactam, allantoin, and β-sitosterol. The identical acid components were later found in *Aristolochia debilis*.[6] The new acid-C was converted to various derivatives and structure I was tentatively proposed by the Japanese workers from the results of elemental analysis, infrared spectrum, and various chemical properties.



I

The total synthesis of the methyl ester methyl ether of I was undertaken to provide unequivocal proof for the postulated structure (see Chart I). We have recently reported the synthesis of several substituted phenanthrenes, including aristolochic acids I and II, by photocyclization of substituted 2-iodostilbenes.[1,7] This general reaction has been used in the present synthesis. The synthetic precursor of ring A of the desired phenanthrene derivative, namely, 2-carbomethoxy-4,5-methylenedioxyphenylnitromethane (XIV) was available from our earlier work.[1] The synthetic precursor of ring C, 2-iodo-4-methoxybenzaldehyde (VI), had been prepared by Hodgson[8] by Reimer–Tiemann reaction on *m*-iodophenol, followed by dimethyl sulfate methylation. These reactions proved unsuitable since the yields obtained seldom exceeded 10%. Our subsequent approach was very

(1) Part X: S. M. Kupchan and H. C. Wormser, *J. Org. Chem.*, **30**, 3792 (1965).

(2) Abstracted from a part of the dissertation submitted by H. C. Wormser to the University of Wisconsin Graduate School, June 1965, in pa l fulfillment of the requirements of the Ph.D. degree.

(3) This investigation was supported in part by research grants m the National Cancer Institute (CA-04500) and the American Cance ociety (T-275).

(4) American Foundation for Pharmaceutical Education Fello 1961–1963; National Institutes of Health Predoctoral Fellow, 1963–19

(5) M. Tomita and S. Sasagawa, *J. Pharm. Soc. Japan*, **79**, 973 (1959).

(6) S. Sasagawa, *J. Pharm. Soc. Japan*, **82**, 921 (1962).

(7) S. M. Kupchan and H. C. Wormser, *Tetrahedron Letters*, 3 (1965).

(8) H. H. Hodgson and T. A. Jenkinson, *J. Chem. Soc.*, 3041 (27).