boiled gently for 5 minutes. The solution was cooled and adjusted to *p*H 1 with dilute hydrochloric acid. A gray flocculent precipitate formed that could be extracted readily with ether. The ether extract was concentrated and the resulting product recrystallized twice from methyl ethyl ketone. The purified product weighed 34.3 mg. and melted with decomposition and evolution of gas between 209 and 220° depending on the rate of heating.

Anal. Calcd. for $C_{13}H_{14}O_5N_2$: C, 56.1; H, 5.65; N, 9.75. Found: C, 56.64; H, 5.20; N, 9.79.

This product gave a purple ferric chloride test, and was soluble in 5% sodium bicarbonate but insoluble in 5% hydrochloric acid solution.

Antimycic Acid Methyl Ester-Methyl Ether (V).—A solution of 192 mg. of II in 4 ml. of methanol and 0.1 ml. of concentrated hydrochloric acid was heated under reflux for 1.5 hours. The reaction mixture was concentrated *in vacuo*, and the colorless oily residue treated with a solution of diazomethane (from 1 g. of nitrosomethylurea) in 25 ml. of ether containing 1 ml. of methanol. After standing at room temperature overnight the solvents were removed, and the yellow oily residue dissolved in 10 ml. of ethylene dichloride and decolorized with charcoal. The material could then be crystallized from ethyl acetate to give a 60% yield of dense colorless needles, m.p. 155.5–156.0°.

Anal. Calcd. for $C_{13}H_{18}O_5N_2$: C, 55.3; H, 6.42; N, 9.92. Found: C, 55.69; H, 6.40; N, 10.06.

Acknowledgment.—We wish to thank Dr. Curt Leben for much indispensable assistance in carrying out fermentations to produce antimycin, and Mr. Gaylord Barlow for numerous antimycin assays. Especial thanks are due Mrs. J. L. Johnson, Mr. H. S. Gutowsky and Dr. D. R. Johnson for measuring and interpreting infrared spectra, and to E. E. van Tamelen for suggestions and advice.

MADISON, WISCONSIN

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AT THE UNIVERSITY OF ROCHESTER]

Colchicine and Related Compounds

BY GORDON A. NICHOLLS[1] AND D. STANLEY TARBELL

RECEIVED AUGUST 1, 1952

Nitration of colchiceine under mild conditions gave a nitro compound which was reduced to the corresponding amine. The amine rearranged to a hydroxycarboxylic acid on treatment with nitrous acid, in the presence of hypophosphorus acid. Hydrogenation of colchicine in benzene using Adams catalyst gave mainly a desmethoxyhexahydrocolchicine isomer, m.p. 174–175°.

The Dewar tropolone structure for colchicine[2] is now regarded as the most likely. The evidence supporting this formula has already been reviewed.[3]

As a disubstituted tropolone, colchiceine would be expected to undergo a number of reactions, now recognizable as typical of the tropolone compounds in general.[3,4] The results of our examination of some previously undescribed substitution reactions of colchiceine are incomplete, but it has been found that colchiceine can be nitrated under mild conditions to give a crystalline mononitro derivative in poor yield. Although this finding is at variance with a recent statement,[5] our results proved to be reproducible and seemingly originated in a different choice of experimental conditions.

The nitro compound was recrystallized from benzene, a sample dried at room temperature analyzing for a compound of empirical formula, $C_{24}H_{25}O_8N_2$, which is in agreement with that required for a mononitrocolchiceine isomer containing one-half mole of solvent of crystallization. The loss of weight and new empirical formula, after prolonged drying, support this contention. Although no crystalline derivative of the nitro compound could be prepared, methylation with methyl sulfate in acetone over anhydrous potassium carbonate gave an amorphous alkali-insoluble product which did not give a color test with ferric chloride. In contrast the nitro compound, like colchiceine, gives a green color with ferric chloride and is soluble in sodium bicarbonate.

The amino compound, prepared in good yield by catalytic reduction of the nitro compound in dioxane, was amphoteric, gave a green color with ferric chloride, but was no longer soluble in sodium bicarbonate. From this information it is possible to conclude that there has been no contraction of ring C in the formation of the nitro compound. No crystalline derivative of the amine was obtained; nevertheless, the amorphous acetate was insoluble in dilute acid and dissolved very slowly in dilute sodium hydroxide. If it was a derivative of colchiceine, reaction of this amine with nitrous acid in hypophosphorous acid would result, either in the replacement of the amino group by hydrogen to give back the colchiceine, or in rearrangement during the course of the reaction to give a salicylic acid derivative, by analogy with the known behavior of *o*-aminotropolones.[6] The product of the reaction of the amino compound with nitrous acid in hypophosphorous acid was not identical with colchiceine. It analyzed for a compound, $C_{21}H_{23}O_7N$, in which the amino groups had been lost and an oxygen atom gained. This compound is readily soluble in aqueous sodium bicarbonate, gives a positive reaction with ferric chloride, and forms a bicarbonate-soluble monoacetate, which no longer gives a ferric chloride test. These facts show that it is a phenolic carboxylic acid and presumably the salicylic acid type rearrangement product I or II, analogous with that obtained from *o*-aminotropolones.[6]

(1) Postdoctoral Fellow of the National Cancer Institute of the National Institutes of Health, Public Health Service, Federal Security Agency, 1950–1952.

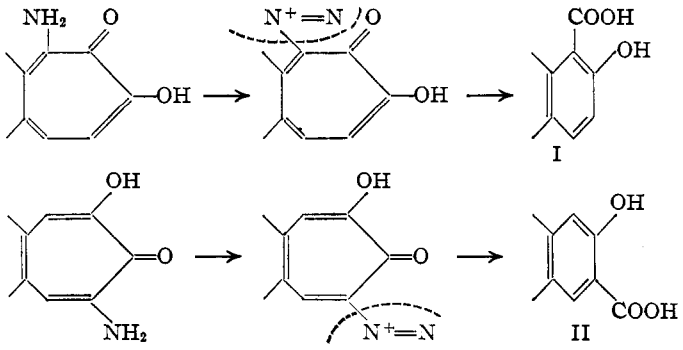
(2) M. J. S. Dewar, *Nature*, **155**, 141, 479 (1945).

(3) J. W. Cook and J. D. Loudon, *Quarterly Rev.*, **5**, 99 (1951).

(4) T. Nozoe, *Nature*, **167**, 1055 (1951).

(5) H. Fernholz, E. Hartwig and J. C. Salfeld, *Ann.*, **576**, 131 (1952).

(6) (a) R. D. Haworth and J. D. Jeffries, *J. Chem. Soc.*, 2067 (1951); (b) T. Nozoe, Y. Kitahara and K. Doi, THIS JOURNAL, **73**, 1895 (1951).



Attempts to obtain more rigid evidence that nitration had occurred by attack on a carbon atom adjacent to the functional groups in ring C were unsuccessful. Permanganate oxidation failed to give N-acetylcolchicinic anhydride.[7]

The infrared spectra of the nitro, amino and hydroxycarboxylic compounds (Fig. 1) are compatible with the above deductions. The intense band near 1620 cm.$^{-1}$ in the nitro and amino compound and its absence in the hydroxy compound, which has instead an intense peak at 1660 cm.$^{-1}$, confirms the view that the first two are tropoloid and the third a carboxylic acid. Additional support is derived from the fact that, although the infrared spectra of all three compounds are very similar in the region 950–1350 cm.$^{-1}$, the hydroxy compound, unlike the other two, has no strong band near 1275 cm.$^{-1}$. This band appears to be a characteristic of the tropolone compounds.[8]

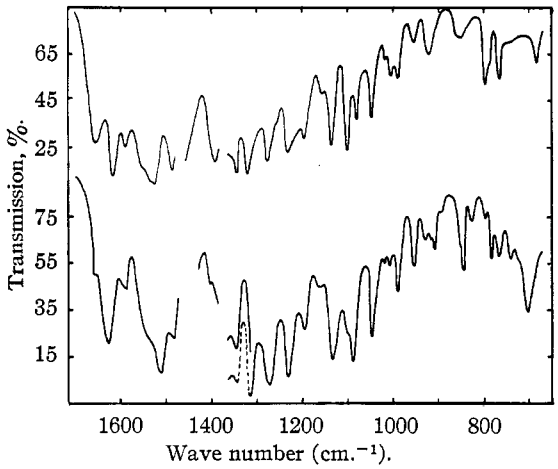


Fig. 1.—Infrared absorption spectra of nitrocolchiceine (upper) and aminocholchiceine.

A previous paper[9] reported the results of some reduction experiments intended to lead to additional evidence in favor of a seven-membered ring C structure for colchicine, by making use of Bursian's hexahydro compound,[10] from which the ring C oxygen atoms had apparently been removed. This compound, however, could not be obtained.

By varying the conditions of hydrogenation we have now found that colchicine in benzene with Adams catalyst gives a 10–15% yield of desmethoxyhexahydrocolchicine,[9,10] m.p. 174–175°, along with a very small amount of a new desmethoxyhexahydrocolchicine, m.p. 126–128°, and hexahydrocolchicine, m.p. near 124°. The identity of the latter compound was confirmed by preparation of the known acetate.[10] This on mild hydrolysis regenerated what is presumed to be the original compound, m.p. near 124°. After drying in an evacuated desiccator at room temperature for three weeks, this same sample melted at 167–167.5° with prior shrinking at 120°. Although we have not been able to raise the m.p. of this isomer without going through the acetate, the compound before this treatment has an infrared spectrum identical with that of the compound, m.p. 167–167.5°, and different from that of the other isomer, m.p. 171.5–172°.

The infrared spectra of the pair of desmethoxyhexahydrocolchicine stereoisomers and the pair of hexahydroxycolchicine stereoisomers were determined. A comparison of the members of each pair of curves shows a striking similarity, but at the same time the separate identity of the isomers is clearly indicated.

It seemed possible that the 174° desmethoxy compound could be used as an intermediate in obtaining Bursian's compound, and perhaps more interesting degradation products. While we were engaged in attempts to remove the hydroxyl group from ring C of this compound, the degradation of colchicine to octahydrodesmethoxydesoxydesacetamidocolchicine was reported by others.[11]

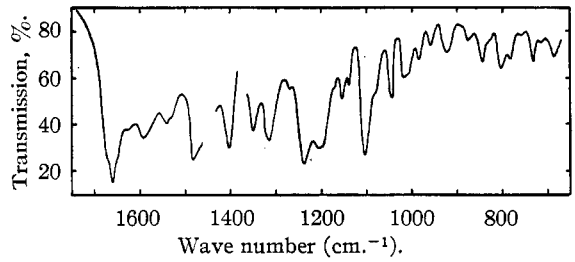


Fig. 2.—Infrared absorption spectrum of the hydroxycarboxylic acid from aminocolchiceine.

Some of the compounds described in this paper have been submitted to Dr. C. Chester Stock, Sloan-Kettering Institute, for testing for tumor-inhibiting properties.

Experimental[12]

Colchicine.[13].—An acetone solution of U.S.P. colchicine (50 g.) was chromatographed on grade I–II alkali free alumina (1.9 × 40 cm.) and eluted with acetone (850 cc.). The gum (47.3 g.) obtained from the eluant, crystallized on seeding and was twice recrystallized from ethyl acetate to give colchicine, m.p. 155–157° (dec.).

Colchiceine.—Colchiceine was prepared in 80–86% yield from colchicine (16.0 g.) by heating (1.75 hr.) in a steam-bath with 0.1 *N* hydrochloric acid (160 cc.). The anhydrous form, obtained from ethyl acetate–hexane, melted at

(7) J. W. Cook, T. Y. Johnston and J. D. Loudon, *J. Chem. Soc.*, 537 (1950).

(8) (a) G. P. Scott and D. S. Tarbell, THIS JOURNAL, **72**, 240 (1950); (b) H. P. Koch, *J. Chem. Soc.*, 512 (1951); (c) G. Aulin-Erdtmann and H. Theorell, *Acta Chem. Scand.*, **4**, 1490 (1950).

(9) A. D. Kemp and D. S. Tarbell, THIS JOURNAL, **72**, 243 (1950).

(10) K. Bursian, *Ber.*, **71**, 245 (1938).

(11) H. Rapoport and A. R. Williams, THIS JOURNAL, **73**, 1896 (1951).

(12) Analyses by Miss Claire King; melting points are uncorrected and were determined using a copper block apparatus.

(13) *Cf.* J. N. Ashley and J. O. Harris, *J. Chem. Soc.*, 677 (1944).

177–179°.[14] The filtrate from the reaction mixture was neutralized with sodium bicarbonate to give a gelatinous precipitate (680 mg.) of trimethylcolchicinic acid, m.p. 163°[15] after crystallization from hot methanol containing a few drops of water.

Nitrocolchiceine.—Initial experiments showed that the reaction of colchiceine with nitric acid is quite sensitive to change of reaction conditions. The best result which was obtained is described below, and has proved to be reproducible. A solution of colchiceine (6.0 g.) in acetic acid (10 cc.) and water (3 cc.) was stirred in an ice-bath while 7 cc. of a solution of nitric acid (2 cc., d^{20}_4 1.41), glacial acetic acid (5 cc.) and water (5 cc.) was added slowly dropwise. After stirring at 0–5° for 1.1 hr. the reaction mixture, which had turned brown, was stirred into ice-cold water (110 cc.), the yellow precipitate collected and washed twice by stirring for 0.5 hr. with cold water (2 × 15 cc.) to give a fine yellow powder (3.6 g.). Several recrystallizations from benzene or benzene–ethanol gave yellow leaflets (1.15–1.50 g.), m.p. indefinite with foaming near 148–150°. No other crystalline product could be isolated. A sample for analysis was dried overnight in a desiccator.

Anal. Calcd. for $C_{24}H_{25}O_8N_2$, *i.e.* $C_{21}H_{22}O_8N_2 \cdot {}^1/_2C_6H_6$: C, 61.40; H, 5.37. Found: C, 61.05, 61.36; H, 5.32, 5.53.

This same sample darkened on drying in a vacuum at 110° over phosphorus pentoxide for 2 days, and analyzed as follows.

Anal. Calcd. for $C_{21}H_{22}O_8N_2$: C, 58.60; H, 5.15; weight loss for ${}^1/_2C_6H_6$, 8.32. Found: C, 58.10; H, 5.29; weight loss, 8.30.

The compound gave erratic nitrogen analyses. It is soluble in aqueous sodium bicarbonate, from which it is recovered unchanged, and gives a green coloration with ferric chloride reagent.

Aminocolchiceine.—A solution of nitrocolchiceine (1.32 g.) in freshly purified dioxane (40 cc.) was added to a suspension of prereduced Adams catalyst (430 mg.) in the same solvent (30 cc.) and reduced with hydrogen at room temperature (25°) and pressure. Hydrogen uptake ceased after an apparent absorption of 3.28 moles in 45 min. The catalyst was removed, the filtrate concentrated to dryness, and the residue purified by recrystallization from hot ethyl acetate and hot ethyl acetate–heptane. The product was obtained in 78–80% yield as yellow prisms, vacuum tube m.p. 269–270° (dec.). The reaction was reproducible.

Anal. Calcd. for $C_{21}H_{24}O_6N_2$: C, 62.99; H, 6.04; N, 7.00. Found: C, 62.64, 63.02; H, 6.23, 6.24; N, 7.25.

The compound is insoluble in aqueous sodium bicarbonate, difficultly soluble in aqueous sodium carbonate and gives a green coloration with ferric chloride reagent.

Reaction of Aminocolchiceine in Hypophosphorous Acid with Nitrous Acid.[16]—A solution of aminocolchiceine (250 mg.) in 30% hypophosphorous acid (2 g.) was stirred at 0–5° while 0.5 cc. of aqueous sodium nitrite (47 mg.) was added dropwise from a capillary. The reaction mixture was left in an ice-bath 1.5 hr. during which time the initial orange precipitate decomposed with effervescence to a pale cream precipitate. This was collected, washed, dried (245 mg.) and recrystallized from cold ethyl acetate–hexane as almost colorless plates, m.p. indefinite with decomposition near 175°. The reaction was reproducible.

Anal. Calcd. for $C_{21}H_{23}O_7N$: C, 62.83; H, 5.78; N, 3.49. Found: C, 62.92; H, 5.60; N, 3.45.

The compound is readily soluble in aqueous sodium bicarbonate and gives a green coloration with ferric chloride reagent.

The acetate was prepared by suspending the compound (50 mg.) in acetic anhydride (0.5 cc.) and adding one drop of 60% perchloric acid from a capillary. After 15 min. at room temperature (25°) the reaction mixture was stirred into cold water (10 cc.), left 4 hr. with frequent scratching, and the crystalline precipitate collected (47 mg.). This was recrystallized with difficulty from cold ethyl acetate–hexane as colorless prisms, m.p. indefinite with decomposition near 190°.

Anal. Calcd. for $C_{23}H_{27}O_9N$, *i.e.*, $C_{23}H_{25}O_8N \cdot H_2O$: C, 59.86; H, 5.90. Found: C, 60.17; H, 5.80 (for sample dried in a vacuum at 110° for 3 hr. over phosphorus pentoxide).[17]

The compound is readily soluble in aqueous sodium bicarbonate, but does not give a color reaction with ferric chloride.

Hydrogenation of Colchicine in Benzene with Adams Catalyst.—A solution of pure colchicine (4.0 g.) in dry sulfur-free benzene (50 cc.) was added to prereduced Adams catalyst (500 mg.) in the same solvent (30 cc.), and hydrogenated at room temperature (25°) and 80 cm. pressure. During 4.5 hr. 3.2 moles of hydrogen was absorbed and after another 20 hr., when 3.8 moles of hydrogen had been absorbed, hydrogen uptake had stopped. The experiment was repeated using identical conditions, the two reaction mixtures combined, the solution warmed to dissolve the colorless crystals which had separated during the hydrogenation, and the catalyst removed. By just saturating the cold benzene filtrate with water and recrystallizing the product from the same solvent, without allowing the water to be removed azeotropically, colorless plates (1.24 g.), m.p. 157–159°*[18] were obtained—*fraction 1.* Over a period of several weeks the combined filtrates (water saturated) were fractionally concentrated and the residues fractionally recrystallized from ethyl acetate (water) to give microcrystalline solids, m.p. 100–120° (2.64 g.)—*fraction 2.* After removal of the solvent under reduced pressure the final ethyl acetate filtrate afforded a resin. This was obtained as an amorphous powder (2.5 g.), m.p. 100°, by triturating with hexane, and could not be crystallized either directly or after chromatographing on alumina.

Fraction 1 was repeatedly crystallized from benzene (water) to give α-desmethoxyhexahydrocolchicine (yield 10–15%), m.p. 174–175° undepressed by a sample of the desmethoxy compound, m.p. 171–172°, described by Kemp and Tarbell.[9]

This compound has also been described by other workers.[10,11]

The acetate (55 mg.) was prepared in 73% yield using the method already described above. Two recrystallizations from ethyl acetate (water) afforded colorless needles, m.p. 212.5–213°. Bursian[10] reports m.p. 210°.

Anal. Calcd. for $C_{23}H_{31}O_6N$: C, 66.17; H, 7.48. Found: C, 66.35; H, 7.60.

After isolating the α-desmethoxy compound, m.p. 174–175°, the residue from the mother liquors, to which was also added a relatively small amount of residue from earlier reductions, was fractionally crystallized from benzene, and the low m.p. fractions recrystallized from acetone (water) and ethyl acetate (water) to give colorless plates of α-desmethoxyhexahydrocolchicine, m.p. 126–128°.*[19]

The acetate (28 mg.) was prepared in 60% yield using the method already described above. Recrystallization from methanol–water gave colorless prisms, m.p. 169–170°.

Anal. Calcd. for $C_{23}H_{31}O_6N$: C, 66.17; H, 7.48. Found: C, 66.58; H, 7.31.

Fraction 2 was repeatedly crystallized as colorless needles from methanol–water and ethyl acetate to give hexahydrocolchicine (700 mg.), m.p. indefinite but near 121–124°.[20] This was the only compound isolated from this fraction.

(14) Meyer and Reichstein, *Pharm. Acta Helv.*, **19**, 27 (1944), report m.p. 178–179.5°.

(15) S. Zeisel, *Monatsh.*, **9**, 17 (1888), reports the m.p. as 156–159°.

(16) The reaction conditions are similar to those used for deamination by N. Kornblum and D. C. Iffland, THIS JOURNAL, **71**, 2137 (1944).

(17) Many of the compounds in this series hold water tenaciously and are hygroscopic when anhydrous. After drying at 130° for 4 days the acetate was slightly discolored and on exposure to air rapidly increased in weight by only 1.45%, presumably because dehydration was still incomplete.

(18) An asterisk is used to indicate that a sample for melting point was dried at 110° over phosphorus pentoxide in a vacuum for 1 hr.

(19) In contrast with the hexahydrocolchicine isomer, m.p. "124°" (see below); β-desmethoxyhexahydrocolchicine, m.p. 126–128°, does not change to a glassy solid when dried at 100°,[9] but retains its crystalline form. The amount of water present in the solvents had a very critical bearing on how readily the desmethoxy isomers could be separated. Too much water gave little or no appreciable separation.

(20) The indefinite character of the m.p. of this compound, which is apparently a characteristic of the compound rather than an indication of its being impure, led to the preparation of a sample for infrared analysis through the acetate.

The acetate was prepared in 58% yield using the method already described above. It was necessary, however, to extract the neutralized aqueous solution with ethyl acetate to obtain the product, which recrystallized readily from ethyl acetate–heptane (1:1) as colorless needles, m.p. 164.5–165°.[21]

The acetate was *hydrolyzed* in methanol (5 cc.) with 15 cc. of 0.047 *N* sodium hydroxide at room temperature. After one day the solution was concentrated by a cold air current, the product collected (140 mg.), recrystallized from methanol–water, and then from ethyl acetate (water) to give colorless needles, m.p. indefinite but near 122–126°. After drying at room temperature for three weeks in an evacuated desiccator this same sample melted at 167–167.5° with prior shrinking at 120°. A sample for infrared analysis was dried further in a vacuum at 110° over phosphorus pentoxide for 4 hr., m.p. 167–167.5°.

Infrared Spectra.—Percentage transmission curves were plotted from sample and solvents tracings obtained by Mr. Carl Whiteman with a Perkin–Elmer single beam recording spectrometer (Model-12A) using a rock salt prism and a 0.025 mm. thick cell. The samples were suspended in Nujol.

(21) K. Bursian (ref. 10) reports m.p. 160°.

ROCHESTER, N. Y.

[CONTRIBUTION FROM THE CHEMICAL RESEARCH DIVISION OF SCHERING CORPORATION]

X-Ray Diagnostics. VII. Cholecystographic Agents[1]

BY DOMENICK PAPA, HELEN F. GINSBERG, ILSE LEDERMAN AND VIRGINIA DECAMP

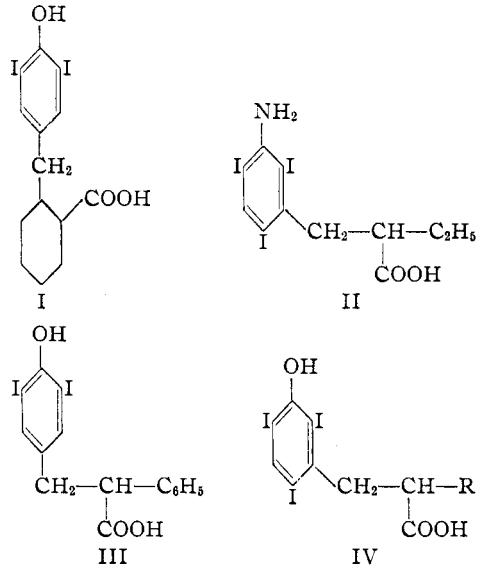
RECEIVED OCTOBER 3, 1952

The preparation of a series of α-alkyl-, α-phenyl- and α-cyclohexyl-β-(polyiodo-*m*-hydroxyphenyl)-propionic acids is described. These compounds have been obtained from *m*-hydroxy- or *m*-nitrobenzaldehyde by previously described series of transformations. Included in this study are the α-ethyl-β-(2,4,6-triiodophenyl)- and α-ethyl-β-(2,3,4,6-tetraiodophenyl)-propionic acids.

Within the last ten years, numerous studies[2] have appeared on the correlation of chemical structure and cholecystographic property. These studies have had as their objective the preparation of a cholecystographic medium combining: (a) complete absorption of the medium from the intestinal tract (no residual dye in colon); (b) high concentration of the medium in the gall bladder (optimal density); and (c) minimal or no systemic toxicity. Although the chemical and patent literature indicates that a relatively large number of compounds fulfill these requirements, only two compounds, I and II,[3] have been introduced into clinical practice since the advent of iodoalphionic acid, α-phenyl-β-(3,5-diiodo-4-hydroxyphenyl)-propionic acid (III).[4]

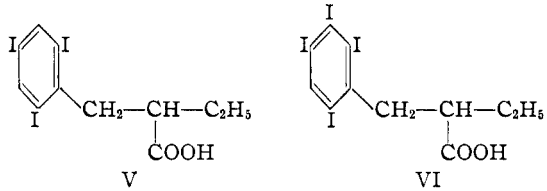
Compounds I and III have in common the diiodohydroxyphenyl group characteristic of many "cholecystographic agents," whereas compound II, embracing the triiodoaminophenyl moiety, represents a departure in this type of X-ray medium. Notwithstanding these differences, the three compounds qualitatively are equivalent in their clinical application, although they differ in degree of density of shadow, extent of systemic toxicity and quantity of residual dye in the colon.

The present study describes the preparation and preliminary pharmacological evaluation of a new series of iodinated compounds of general formula



I II III IV

IV, as well as substances V and VI. It is to be noted that compounds of general formula IV are



V VI

the hydroxy analogs of the substance of formula II, whereas compounds V and VI have neither a hydroxyl nor an amino group and, in this respect, are similar to several substances previously described,[5] for which no pharmacological data have been reported.

The compounds of general formula IV were prepared as shown in the equation

(1) Presented in abstract before the Division of Medicinal Chemistry, American Chemical Society Meeting, Atlantic City, N. J., September 15, 1952.

(2) (a) S. Natelson, B. Kramer and R. Tekel, U. S. Patent 2,400,433 (May 14, 1946); (b) E. Schwenk and D. Papa, U. S. Patent 2,436,270 (February 17, 1948); (c) B. S. Epstein, S. Natelson and B. Kramer, *Am. J. Roentgenol.*, **56**, 201 (1946); (d) D. Papa, *et al.*, THIS JOURNAL, **72**, 2619, 2623, 4906, 4909 (1950); **73**, 253 (1951); (e) S. Archer, *et al.*, *ibid.*, **71**, 3749, 3753 (1949); *J. Am. Pharm. Assoc. Sci. Ed.*, **40**, 143, 617 (1951) and others.

(3) Compound I is "Monophen" of National Synthetics (S. Natelson, B. Kramer and R. Tekel, U. S. Patent 2,496,064 (January 31, 1950)) and II is "Telepaque" of Winthrop Stearns.[2e]

(4) M. Dohrn and P. Diedrich, U. S. Patent 2,345,384 (March 28, 1944).

(5) J. W. Barnett, F. A. Robinson and B. M. Wilson, *J. Chem. Soc.*, 202 (1947), and E. Schwenk and D. Papa, U. S. Patent 2,436,270 (February 17, 1952).