[CONTRIBUTION FROM THE RESEARCH DIVISION, SMITH, KLINE AND FRENCH LABORATORIES]

Colchicine. Some Reactions of Ring C[1]

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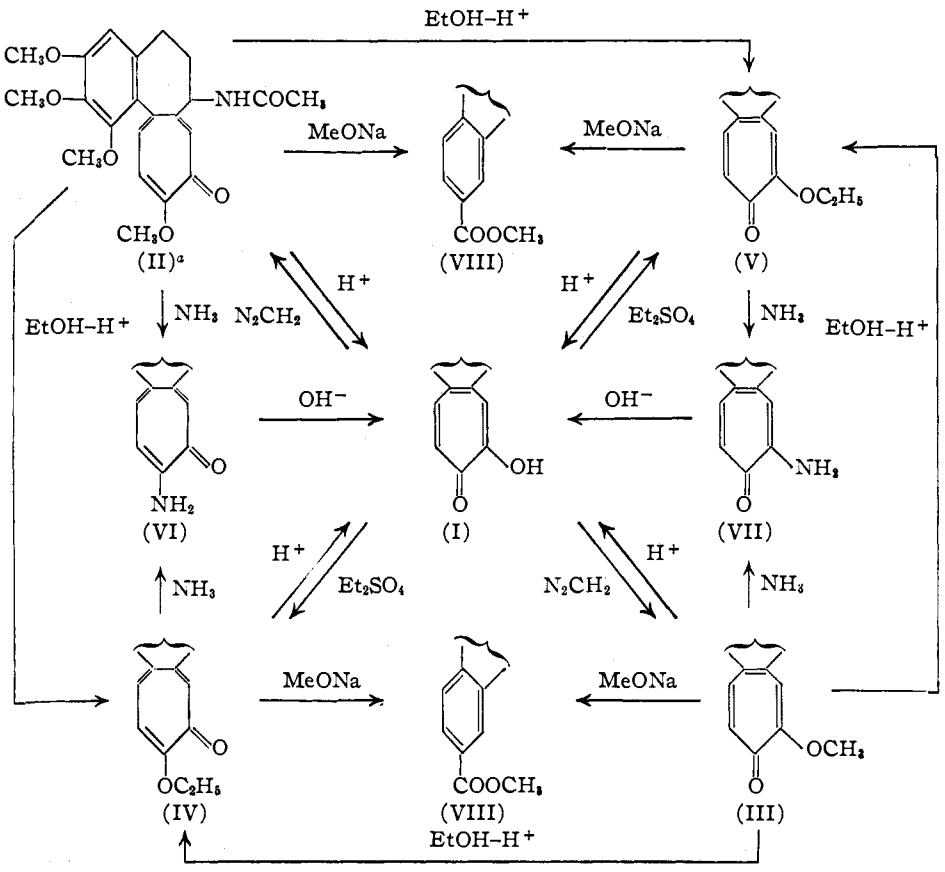
The isomeric methyl ethers and isomeric ethyl ethers of colchiceine have been related by means of physical properties and by conversion to the amides of colchiceine. The preparation of the unknown members of this series is described. It is shown that either of the methyl ethers may be converted to a mixture of the ethyl ethers by ethanolysis under acidic conditions. A possible mechanism for this change is discussed. The absorption spectra of the various compounds are presented and several significant features are pointed out.

The formulation of ring C of colchicine as a derivative of tropolone[3] appears to be compatible with the known chemical and physical data, although a decisive proof of structure has yet to appear.[4] While some information on the ethers of colchiceine and their transformation products is available, the interrelationships between these compounds have not been completely elucidated. Our interest in the effect of structural variation on biological activity led us to undertake a study of some of these relationships. The data now at hand have been interpreted by means of the tropolone formulation and are summarized in Scheme I.

Methylation of colchiceine (I) with diazomethane gives a mixture of isomers which was first separated by Sorkin[5] into colchicine (II) and the previously unknown isocolchicine (III). Similarly, ethylation of colchiceine with ethyl sulfate and alkali yields an amorphous solid which has been separated by chromatography into two isomeric, crystalline ethyl ethers.[6] All of the ethers give colchiceine on hydrolysis, although colchicine appears to hydrolyze more readily than the others. One of the ethyl ethers (V), obtained by ethylating colchiceine with diazoethane, has been recently reported by Santavy and Reichstein,[7] who assigned to it the iso structure by comparison of its ultraviolet absorption spectrum and optical rotation with those of colchicine and isocolchicine. As may be seen from Table I, the iso series shows a greater specific rotation than does the "normal" series and the position of the longer wave length absorption band of the iso ethers is shifted about 8 mμ toward the violet.

Since the methoxyl group of ring C may be regarded as vinylogous with the methoxyl group of



SCHEME I

I = Colchiceine
II = Colchicine[a]
III = Isocolchicine
IV = Colchiceine ethyl ether
V = Isocolchiceine ethyl ether
VI = Colchiceine amide
VII = Isocolchiceine amide
VIII = Colchicic acid methyl ester

[a] The structure shown for colchicine is that given by King and Pepinsky on the basis of X-ray diffraction studies (Abstracts of papers, 119th Meeting of the American Chemical Society, 1951, p. 33C).

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(3) Dewar, *Nature*, **155**, 141 (1945).

(4) (a) Loudon, *Ann. Rept.*, **45**, 187 (1948); (b) Abstract of a "Symposium on Tropolone and Allied Compounds," Chemistry and Industry, 1951, pp. 12, 28; (c) Doering and Knox, THIS JOURNAL, **73**, 828 (1951).

(5) Sorkin, *Helv. Chim. Acta*, **29**, 246 (1946).

(6) Lettré and Fernholz, *Z. physiol. Chem.*, **278**, 175 (1943), reported the preparation of an amorphous mixture of ethyl ethers.

(7) Santavy and Reichstein, *Helv. Chim. Acta*, **33**, 1606 (1950).

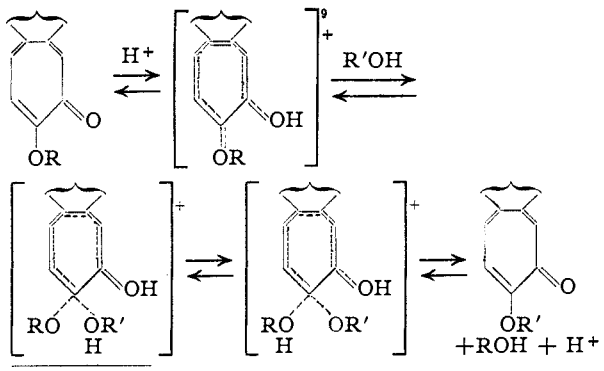
TABLE I

	M.p., °C	$[\alpha]_D$ (c, 1 in chloroform)	t, °C.	Absorption bands in 95% ethanol λ(log E)	λ(log E)	λ(log E)
Colchicine	143.5–148	−123.4	17	350.5 (4.22)	243 (4.47)	
	155–158.5[a]	−118.9	25			
Colchiceine ethyl ether	135–139[b]	−129.4	25	351 (4.21)	243.5 (4.45)	233 (4.44)[c]
Isocolchicine	222–223[d]	−297.6	25[d]	343 (4.28)	243.5 (4.44)	
Isocolchiceine ethyl ether	222–223[e]	−294.5	25[e]	343 (4.27)	246 (4.48)	229 (4.41)
Colchiceine amide	258.5–259.5	−134.0	25	354.5 (4.29)	246 (4.48)	232 (4.37)[c,f]
				370.5 (4.30)[f]	252 (4.53)[f]	
Isocolchiceine amide	159–162[g]	−273.1	25	353.5 (4.30)	247 (4.47)	
				373 (4.26)[f]	252 (4.51)[f]	
Colchiceine	177–178	−255.1	25	351 (4.28)	244 (4.51)	

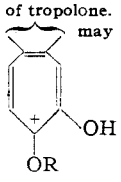
[a] From ethyl acetate–ether; m.p. is of vacuum-dried sample on Kofler stage; the sample appeared entirely melted at 143.5–148°, but in polarized light a few crystals were visible which melted at 155–158.5°. [b] From ethyl acetate–ether on the Kofler stage. [c] Shoulder. [d] Reported[5] m.p. 225–226°; $[\alpha]^{12}D$ −306.7° (c, 1.063 in $CHCl_3$). [e] Reported[7] m.p. 215–218°, 223–225°; $[\alpha]^{19}D$ −293.7° (c, 0.623 in $CHCl_3$). [f] In 0.1 N HCl. [g] Kofler stage.

a methyl ester it was of interest to observe the effect of conditions which, with esters, would ordinarily bring about ester interchange. When colchicine was refluxed for 1.5 hours in ethanol containing *p*-toluenesulfonic acid colchiceine ethyl ether (IV) was isolated in *ca.* 40% yield along with a small quantity (5%) of isocolchiceine ethyl ether (V) and unreacted colchicine. A longer period of refluxing (6 hours) caused a slight increase in the amount of isocolchiceine ethyl ether relative to colchiceine ethyl ether (approximately 1:4) and probably led to side reactions, since it was difficult to obtain the ethers pure. The alcoholysis of isocolchicine appeared to take place at about the same rate as that of colchicine: after a 1.5 hour period of refluxing, a mixture of starting material and isocolchiceine ethyl ether was isolated, while heating for 5.5 hours gave a 34% yield of isocolchiceine ethyl ether (isolated). There was also an indication of the presence of a small amount of colchiceine ethyl ether.

The fact that structural isomerism appears to be largely (though not entirely) preserved during ethanolysis of the isomeric ethers suggests that the reaction occurs mainly through a direct displacement of methanol by the entering ethoxyl group. One of a number of possible formulations for the sequence is as follows[8]



(8) Compare reference 4(c), for an analogous proposal dealing with the etherification of tropolone.

(9) To which  may be regarded as making an important contribution.

An alternate path would be the etherification of a common intermediate, *i.e.*, colchiceine, which might be present in small concentration from hydrolysis of the ethers by traces of water. The operation of this mechanism would account for the formation of mixtures of ethers. An experiment to test this possibility showed that colchiceine, in the presence of ethanol, *p*-toluenesulfonic acid and benzene, can be ethylated in very low yield.[10] It appears, therefore, that the data are best accommodated by assuming the *trans*-etherification goes mainly by way of a direct displacement reaction and to a lesser extent by hydrolysis to and re-etherification of colchiceine.

In contrast to the behavior in acidic alcohol it has been shown[11] that methanol containing a catalytic amount of sodium methoxide causes the C-ring of both colchicine and isocolchicine to become benzenoid with resultant formation of colchicic acid methyl ester (VIII).[12] Under these conditions either of the ethyl ethers can also be converted to VIII. This, incidentally, provides additional evidence that the alkoxyl portion of the colchicic ester originates in the alkoxide reagent used to initiate the reaction; possible mechanisms have been discussed previously.[11b,4c]

According to Zeisel[13] the methoxyl group in ring C of colchicine is replaced by an amino group upon treatment with alcoholic ammonia at 100° to give colchiceine "amide" (VI).[14] This transformation can also be effected in high yield by treating colchicine (II) or the corresponding colchiceine ethyl ether (IV) with concentrated aqueous ammonia at room temperature. Isocolchicine (III) or isocolchiceine ethyl ether (V) afford the isomeric isocolchiceine amide (VII). Both amides are hydrolyzed to colchiceine in hot aqueous alkali.[13] An experiment in which colchiceine amide was hydrolyzed in acid did not yield well-defined products.

(10) Johanny and Zeisel, *Monatsh.*, **9**, 865 (1888), report evidence indicating that the interaction of colchiceine and methanol saturated with HCl gives a low yield of colchicine.

(11) (a) Santavy, *Helv. Chim. Acta*, **31**, 821 (1948); (b) Fernholz, *Ann.*, **568**, 63 (1950).

(12) This substance is variously referred to as colchicic acid methyl ester,[11a] allcolchicine,[11b] colchicine acid methyl ester[7] and methyl colchicicate.[4c] It has been related to the colchinol series by conversion to N-acetylcolchinol.[11b]

(13) Zeisel, *Monatsh.*, **9**, 1 (1888).

(14) The compound has been otherwise named N-acetyltrimethylcolchicinamide[4c] and colchicamide.[11] The latter name might properly refer to the amide of colchicic acid.

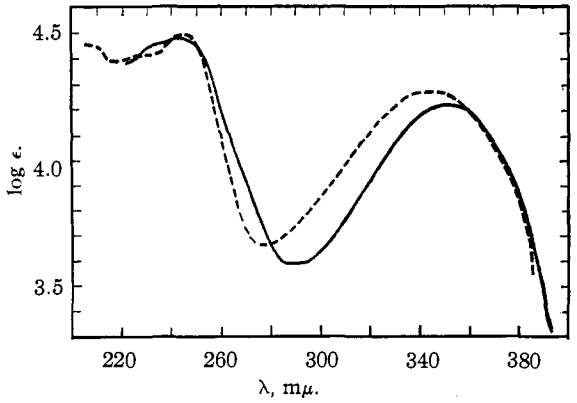


Fig. 1.——, colchicine in 95% ethanol, 3.35 × 10^{-5} *M*; - - - -, isocolchicine in 95% ethanol, 3.38 × 10^{-5} *M*.

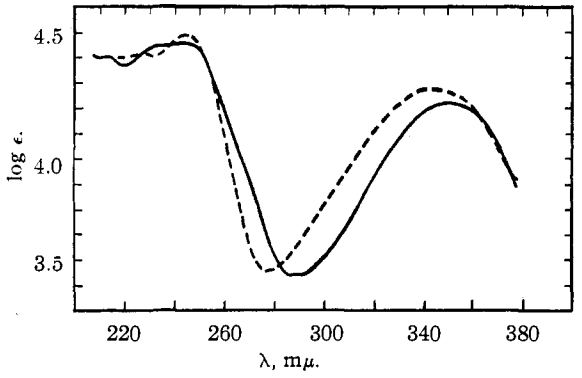
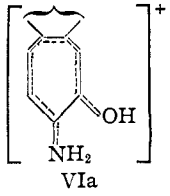


Fig. 2.——, colchiceine ethyl ether in 95% ethanol, 3.43 × 10^{-5} *M*; - - -, isocolchiceine ethyl ether in 95% ethanol, 3.60 × 10^{-5} *M*.

The ultraviolet absorption spectra of the various ethers and amides are shown in Figs. 1–3. It will be noted that the spectra of the isomeric amides show only slight differences, in contrast to those of the isomeric ethers. The bathochromic shift of the longer wave length band of the amides in acid solution can probably be attributed to formation of the conjugate acid VIa. It is not unlike an observation of Doering and Knox[4c] for the spectrum of tropolone in acid solution.



VIa

The infrared spectra of colchicine, colchiceine, trimethylcolchicinic acid and a number of simpler tropolones have been discussed by Scott and Tarbell.[15] The absorption spectra of the amides (VI and VII) from 2 to 15.5 μ are given in Fig. 4. In general, these show much similarity to the spectrum of colchiceine. A comparison of the absorption characteristics for compounds I–VII (incl.) reveals certain consistent differences between the normal and iso series in the vicinity of 7 μ. In this region the normal compounds show more fine structure and have at least one more absorption band than do the iso compounds. This may be seen by reference to Fig. 5.

(15) Scott and Tarbell, THIS JOURNAL, **72**, 240 (1950).

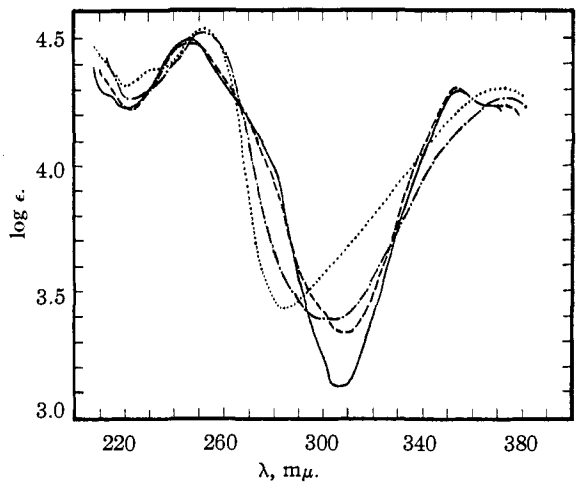


Fig. 3.— —, colchiceine amide in 95% ethanol, 3.21 × 10^{-5} *M*; . . ., colchiceine amide in 0.1 *N* HCl, 3.21 × 10^{-5} *M*; - - - -, isocolchiceine amide in 95% ethanol, 3.33 × 10^{-5} *M*; —·—·—, isocolchiceine amide in 0.1 *N* HCl, 3.33 × 10^{-5} *M*.

Included in Fig. 5 is the spectrum of colchiceine in the 7 μ region. It bears a close resemblance to the spectra of the iso series. This observation, coupled with the fact that colchiceine has a high optical rotation (−255.1° at 25° in chloroform) suggests that colchiceine is a single species, essentially non-tautomeric and belonging to the iso series. It has been postulated that, in tropolone itself, the hydroxylic hydrogen oscillates rapidly between the two adjacent oxygen atoms, so that, on a time

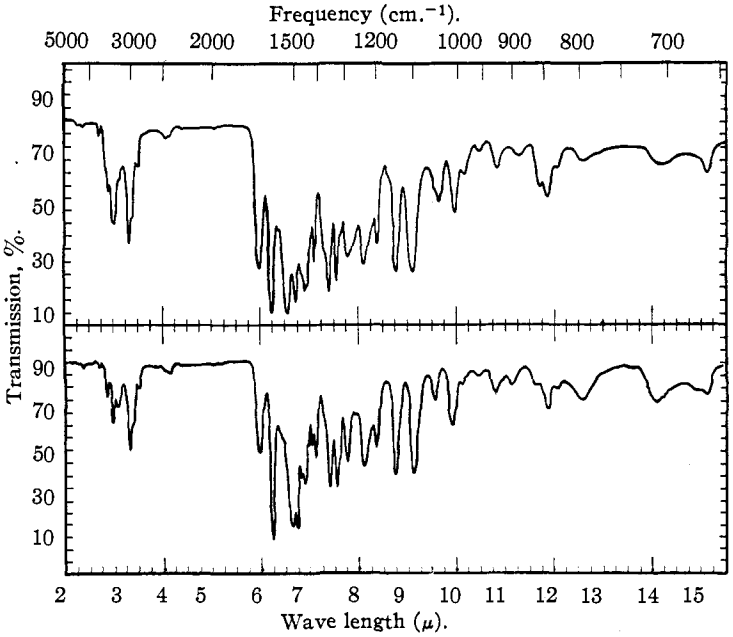


Fig. 4.—Upper curve, isocolchiceine amide; lower curve, colchiceine amide.

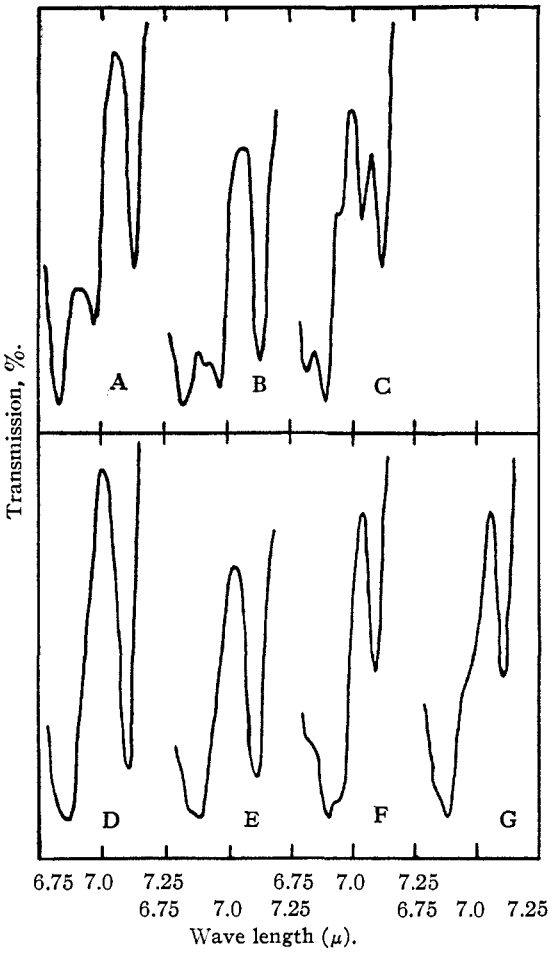
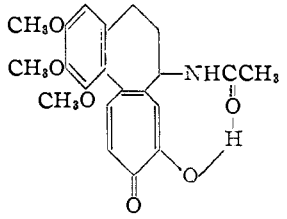


Fig. 5.—A, colchicine; B, colchiceine ethyl ether; C, colchiceine amide; D, isocolchicine; E, isocolchiceine ethyl ether; F, isocolchiceine amide; G, colchiceine.

average basis, it may be regarded as symmetrically located.[16] In colchiceine (or, more properly, isocolchiceine) it is not unlikely that the hydroxylic hydrogen is stabilized at the iso position by hydrogen bonding to the carbonyl oxygen of the acetylamino group



Models show, in at least one configuration of the molecule, that the groups are very favorably situated for this type of interaction.

Acknowledgments.—We should like to express our thanks to Dr. S. David Bailey and Dr. Howard Dinsmore for obtaining and discussing with us the infrared and countercurrent data. We are indebted to the Misses Ruth Savacool, Frances McCarron, Rita Fox and Marguerite Burke for the analytical data and Mr. Arnold Krog for preparing the graphs.

(16) Koch, *J. Chem. Soc.*, 512 (1951).

Experimental

Ethylation of Colchiceine.—A refluxing solution of colchiceine (5.4 g.; 0.014 m.) in 95% ethanol (50 cc.) was treated with small alternate portions of ethyl sulfate (27.6 cc.) and aqueous sodium hydroxide (8.4 g. in 30 cc. of water). The solution still gave a positive ferric chloride test for colchiceine. Sodium carbonate was added and the mixture was evaporated *in vacuo* to half its original volume. It was cooled and extracted with chloroform and the chloroform solution was washed with 10% sodium hydroxide solution. (Acidification of the sodium carbonate solution yielded unreacted colchiceine (1.7 g.).) Evaporation of the chloroform gave a brown gum (3.66 g.), which was dissolved in chloroform–ether (1:1) and adsorbed on alumina. Elution with the same solvent mixture gave an amber colored gum a; elution with chloroform gave a pale yellow gum b.

Colchiceine Ethyl Ether (IV).—Fraction a was dissolved in equal parts of benzene–chloroform–hexane and was placed in the refrigerator. After several weeks large prisms appeared. These were recrystallized from chloroform–petroleum ether, from dioxane–ether (needles, m.p. 157–168° in a capillary, retaining dioxane even after prolonged drying in a vacuum at elevated temperatures), and from ethyl acetate–ether (needles, m.p. 135–139° on the Kofler stage). The compound, as obtained from the latter solvent mixture, was shown to be homogeneous by distribution between ethyl acetate and water in a nine-stage countercurrent distribution apparatus[17] ($[\alpha]^{25}D$ −129.4°; *c*, 1 in chloroform).

The compound (70 mg.) in 0.2 *N* hydrochloric acid (3 cc.), heated on the steam-bath 90 minutes, yielded large needles. These were recrystallized from dioxane–ether to give a white solid (45 mg.) melting at 178–179°; a mixture with colchiceine showed no melting point depression. When the heating period was reduced to 30 minutes the starting material was recovered largely unchanged.

Isocolchiceine Ethyl Ether (V).—Fraction b crystallized readily from warm ethyl acetate. Recrystallization from chloroform–ether gave pale yellow prisms (1.3 g.), m.p. 222–223°.

Anal. Calcd. for $C_{23}H_{27}O_6N$: C, 66.81; H, 6.58. Found: C, 66.48, 66.48; H, 6.58, 6.58.

Hydrolysis to colchiceine (m.p. 173–174°) was effected by dissolving the compound (0.1 g.) in 1 *N* hydrochloric acid (5 cc.)–95% ethanol (5 cc.) and heating on the steam-bath for 90 minutes. A small amount of the starting material was recovered unchanged.

Ethanolysis of Colchicine. A.—A solution of chromatographed[18] colchicine (3.25 g.), *p*-toluenesulfonic acid monohydrate (1.5 g.) and commercial absolute ethanol (550 cc.) was boiled under reflux for 1.5 hours. The solution was evaporated to dryness *in vacuo*. The residue was taken up in chloroform and washed twice with 5% sodium carbonate solution and once with water. Evaporation gave a gum which crystallized in chloroform–petroleum ether, but not readily. It was accordingly dissolved in chloroform (25 cc.) and chromatographed on alumina (16 × 1 cm., Merck). Elution of the column with chloroform (170 cc.) afforded a gum which was crystallized in chloroform–petroleum ether. Recrystallization from dioxane–ether gave a total of 1.9 g. Two further recrystallizations from a mixture of purified dioxane (50 cc.) and ether (100 cc.) afforded long, slender yellow needles (1.6 g.); m.p., after drying at 110° *in vacuo*, 155–165° (softening from 145°). This material analyzed incorrectly for $C_{23}H_{27}O_6N$. The impurities appeared to be dioxane (shown by infrared analysis) and colchicine (shown both by infrared and countercurrent distribution data). The distribution data indicated 1 part of colchicine to 3 parts of colchiceine ethyl ether.[19] Final purification was effected by three recrystallizations from ethyl acetate–ether, which gave pale yellow needles of colchiceine ethyl ether, m.p. 134–138° (Kofler stage) ($[\alpha]^{25}D$ −132.9°; *c*, 1 in chloroform).

Anal. Calcd. for $C_{23}H_{27}O_6N$: C, 66.81; H, 6.58. Found: C, 66.54; H, 6.79.

The combined liquors yielded 0.18 g. of somewhat impure isocolchiceine ethyl ether, m.p. 207–216°.

(17) Raymond, *Anal. Chem.*, **21**, 1292 (1949).

(18) Ashley and Harris, *J. Chem. Soc.*, 677 (1944).

(19) Data to be published.

B.—A solution of chromatographed colchicine (6.37 g.), commercial absolute ethanol (800 cc.) and *p*-toluenesulfonic acid monohydrate (1.46 g.) was boiled under reflux for 6 hours, during which 90 cc. of distillate was removed. The gum remaining after evaporating the solvent *in vacuo* was taken up in chloroform (160 cc.) and was passed through a column of alumina (25 × 2 cm.) to remove toluenesulfonic acid. The acid-free product was dissolved in chloroform–ether (1:1) (120 cc.) and was adsorbed onto a column of alumina (58 g., 21 × 2 cm.) prepared in the same solvent mixture. The column was eluted with chloroform–ether, chloroform and methanol–chloroform. A total of 37 fractions were collected, most of which yielded mixtures of solids from dioxane–ether. Mechanical separation of the crystals gave two fractions, *a* and *b*.

Fraction a melted at 223.5–224.5° (from dioxane–ether). The mixed melting point with isocolchiceine ethyl ether (V), prepared by ethylating colchiceine, was not depressed.

Fraction b was obtained as pale yellow needles from dioxane–ether. The substance melted at 160–170° with frothing. Another sample of colchiceine ethyl ether, crystallized from the same solvent, had the same appearance and melted over the same range. The melting range was not affected by mixing the compounds.

The various fractions were recombined to give a mixture of substances having $[\alpha]^{25}D$ −167.0° ($CHCl_3$). Assuming the mixture to consist chiefly of the ethyl ethers, it may be computed that the ratio of colchiceine ethyl ether to isocolchiceine ethyl ether is approximately 4:1.

Ethanolysis of Isocolchicine.—A mixture of isocolchicine (125 mg.), *p*-toluenesulfonic acid monohydrate (60 mg.) and commercial absolute ethanol (22 cc.) was boiled under reflux for 5.5 hours. Chloroform (22 cc.) was added and the solution passed down a column of alumina (4 × 1 cm.) to remove toluenesulfonic acid. The column was washed with the same solvent mixture (10 cc.) and the combined filtrates were evaporated to dryness. The residual gum was crystallized twice from ethyl acetate–ether to give isocolchiceine ethyl ether (V) (44 mg.), m.p. 220–221°. A mixed melting point with V prepared by ethylating colchiceine was not depressed. The liquors yielded a few milligrams of solid melting at 135–160° from ethyl acetate–ether; this probably contained colchiceine ethyl ether.

Ethylation of Colchiceine with Ethanol.—A solution of colchiceine (2.61 g. m.p. 175–177°), absolute ethanol (485 cc.), *p*-toluenesulfonic acid monohydrate (2.0 g.) and benzene (62 cc.) was boiled under reflux for 11 hours. A total of 200 cc. of distillate was removed during the refluxing. The alcohol was distilled *in vacuo* and the residue taken up in 5% aqueous sodium carbonate (100 cc.). This was extracted with chloroform (3 × 50 cc.). Evaporation of the chloroform gave a small amount of gum which was stirred with tepid water (4 × 20 cc.). Aqueous sodium carbonate (100 cc. of 5%) was added to the water extract and the latter was then re-extracted with chloroform. The material present in the chloroform was transferred to chloroform–ether (1:1) and was adsorbed onto alumina (12 × 1 cm.). Elution with chloroform–ether and chloroform gave a gum which crystallized in ethyl acetate–ether as a pale yellow solid (100 mg.). This melted at 125–210° and gave a color with aqueous ferric chloride only after heating for several minutes in acid solution. It follows that its identity was that of a mixture of the ethyl ethers of colchiceine.

Colchicic Acid Methyl Ester (VIII).—The ether of colchiceine (II, III, IV or V) (50 mg.) was dissolved in absolute methanol (1 cc.) containing sodium methoxide from *ca.* 1 mm.[8] of sodium. The solution was heated just to boiling and then was allowed to stand overnight. Dilution with cold hydrochloric acid gave a solid which was washed with bicarbonate and recrystallized from methanol–water. Yields averaged 50%; m.p. 252.5–253° (capillary). (The reported melting points are 261–262°[11a] and 248°[11b]; $[\alpha]^{20}D$ −141.72° in $CHCl_3$.[11a])

Anal. Calcd. for $C_{22}H_{25}O_6N$: C, 66.13; H, 6.31; CH_3O, 31.08. Found: C, 66.12; H, 6.50; CH_3O, 30.87; $[\alpha]^{25}D$ −140.6° ($CHCl_3$).

Colchiceine Amide (VI). A.—A solution of colchicine·$2CHCl_3$ (5.0 g.) in water (30 cc.) was warmed on the steam-bath for 20 minutes to remove chloroform. To the cooled solution was added 27–29% aqueous ammonia (300 cc.) and this was kept for 18 hours at room temperature. Evaporation to dryness under vacuum gave a gum which was taken up in chloroform (150 cc.) and washed with 1 *N* aqueous sodium hydroxide (2 × 20 cc.) and then with water. After partially evaporating the chloroform and adding ether the product crystallized as a light yellow solid, m.p. 255–256° (4.2 g.). It was recrystallized several times from chloroform (50 cc.) to which was added an equal volume of ether. Yellow prisms were obtained melting at 258.5–259.5°. The compound retained chloroform tenaciously and required 6 hours at 155° *in vacuo* to reach constant weight.

Anal. Calcd. for $C_{21}H_{24}N_2O_5$: C, 65.61; H, 6.29; N, 7.29; CH_3O, 24.21. Found: C, 65.30; H, 6.38; N, 7.38; CH_3O, 24.18.

B.—Colchiceine ethyl ether (50 mg.), dissolved in methanol (1 cc.) and treated with 27–29% aqueous ammonia at room temperature for 20 hours, gave colchiceine amide, m.p. 256–257°.

Colchiceine amide darkens alcoholic ferric chloride; the color disappears on the addition of acid. Nitrous acid gives a deep violet color which rapidly turns brown and then fades to yellow.[13]

Hydrolysis to colchiceine was effected by heating a mixture of colchiceine amide (47 mg.) and 1 *N* sodium hydroxide (2 cc.) for 1 hour. The resulting solution was acidified and the solid recrystallized from dioxane–ether (m.p. and mixed m.p. with colchiceine 175.5–177.5°). An attempt to hydrolyze the amide in acid gave, after 2 hours of heating in 30% sulfuric acid, unchanged amide along with a tarry material.

Isocolchiceine Amide (VII). A.—Isocolchicine (1.16 g.) dissolved in methanol (5 cc.) was added to 27–29% aqueous ammonia (125 cc.). The solution was seeded with isocolchiceine amide (obtained in a previous run which crystallized spontaneously) and was kept at room temperature for 48 hours. The shiny yellow plates (0.8 g.) so obtained were collected, dissolved in methanol and filtered. The filtrate was diluted with water and was evaporated on a hot-plate until the methanol had distilled out and the residual volume was 25 cc. Addition of a seed of isocolchiceine amide resulted in the formation of large yellow plates (0.77 g. after drying at 40°). The melting range after drying for 6 hours *in vacuo* at 140° was 159–162° (Kofler stage). Partial evaporation of the original ammoniacal liquors yielded an additional quantity (0.3 g.) of isocolchiceine amide.

Anal. Calcd. for $C_{21}H_{24}N_2O_5$: C, 65.61; H, 6.29. Found: C, 65.64; H, 6.57.

B.—Isocolchiceine ethyl ether (100 mg.), dissolved in ethanol (2 cc.) and treated with 27–29% aqueous ammonia for 24 hours at room temperature, gave isocolchiceine amide (60 mg.) (m.p. 155–160° in a capillary).

Isocolchiceine amide gives the same color reactions as colchiceine amide. Unlike the latter compound it cannot be recrystallized easily from chloroform–ether.

Isocolchiceine amide (47 mg.) was placed in 1 *N* sodium hydroxide (1 cc.) and the mixture was heated on the steam-bath until the solid dissolved (2 hr.). Addition of concentrated hydrochloric acid gave a precipitate which was extracted with chloroform and transferred to dioxane. Small increments of ether were added until all of the dark, gelatinous material had precipitated. This was filtered out through super-cel and more ether was added. The light solid thus obtained melted at 176–177°; a mixed melting point with authentic colchiceine was not depressed.

Isocolchicine.[5]—A solution of colchiceine (18.0 g.) in methylene chloride (200 cc.) was treated with diazomethane (prepared from 12.0 g. of N-nitrosomethylurea) in the same solvent (120 cc.). After 15 minutes at room temperature the solvent was evaporated *in vacuo* and the residue was dissolved in chloroform–ether (2:1, 250 cc.) and was adsorbed onto alumina (26 × 2 cm.). Elution with chloroform–ether, chloroform and chloroform–methanol led to a partial separation of isomers. Fractions containing isocolchicine were chromatographed twice more on alumina to effect a complete separation. A total of 6.2 g. of isocolchicine was obtained. The product was recrystallized from ethyl acetate–ether containing a small amount of chloroform; m.p. 221.5–222.5°. Countercurrent distribution experiments in a nine-stage apparatus[17] showed the material to be homogeneous with a distribution coefficient between ethyl acetate and water of 0.63. The product, dried to constant weight at 155°, gave $[\alpha]^{25}D$ −297.6° (*c*, 1 in chloroform).

Hydrolysis to colchiceine was effected by heating isocolchicine (50 mg.) in 0.2 *N* hydrochloric acid (2 cc.) for 5

hours on the steam-bath. The resulting needles (34 mg.) were recrystallized from dioxane–ether to give colchiceine; m.p. and mixed m.p. 175.5–177°.

Ultraviolet spectra were determined with a Cary recording quartz spectrophotometer, model 11M. The *infrared spectra* were measured with a Perkin–Elmer double beam spectrophotometer, model 21, either in chloroform solution, or as a glassy film obtained by evaporating a chloroform solution on a rock salt window.

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[CONTRIBUTION FROM THE STERLING-WINTHROP RESEARCH INSTITUTE]

Derivatives of 4-Amino-2-hydroxybenzoic Acid. II

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A series of 2-alkoxy-4-amino- and 4-alkylaminobenzoic acids has been utilized for the preparation of new local anesthetics. The size of the 2-alkoxy group varied from methoxy to *n*-hexyloxy; a number of very highly active local anesthetics were discovered among these compounds.

A previous communication[1] from these laboratories described a number of dialkylaminoalkyl 4-amino- and 4-alkylamino-2-hydroxybenzoates and dialkylaminoalkyl-4-amino- and 4-alkylamino-2-benzyloxybenzoates, prepared as part of a continuing investigation of new local anesthetics. In the present report this series is extended to include basic esters derived from 2-alkoxy-4-aminobenzoic acids and 2-alkoxy-4-alkylaminobenzoic acids.

Several position isomers of the compounds presently discussed have appeared in the literature. A German patent[2] covers a number of dialkylaminoalkyl 3-alkoxy-4-aminobenzoates and 4-alkoxy-3-aminobenzoates. The latter type of isomer has also been investigated by other workers.[3] The diethylaminoethyl 3-amino-4-propoxy- and 4-butoxybenzoates have been reported[3c] to possess a "low anesthetic index" in relation to procaine because of their high subcutaneous and intravenous toxicity. Both compounds produced slight erythema and edema on the human skin.[3c] The corresponding 4-ethoxy compound was described as "suitable for clinical trial,"[3c] although a side chain isomer[3a] was only about one-fifth as active topically as cocaine.

The compounds prepared in the present work were readily synthesized from the parent 2-alkoxy-4-nitrobenzoic acids. 2-Methoxy-4-nitrobenzoic acid has been prepared by several workers,[4] and since the completion of the present work Goldstein and Brochon[5] have described 2-ethoxy-4-nitrobenzoic acid and several of its derivatives. In each case these compounds were prepared by rather difficult and lengthy procedures.

The alkylation of 2-hydroxy-4-nitrobenzoic acid or of an alkyl 2-hydroxy-4-nitrobenzoate by means of an alkyl arylsulfonate in xylene solution, in the presence of potassium carbonate, gave nearly quantitative yields of alkyl 2-alkoxy-4-nitrobenzoates. These in turn could be saponified in very high yield to the free acids by means of an aqueous alcoholic sodium carbonate solution.

The alkylation experiments were of some interest because of the two opposing factors probably present during these syntheses: the solubilizing effect of an alkyl ester grouping on the potassium phenolate, and the steric opposition of this alkyl ester group to the entrance of a second alkyl group in the ortho position. The color changes observed during the alkylation of 2-hydroxy-4-nitrobenzoic acid indicated that alkylation proceeded from the initial ester formation (cream colored) to ester–potassium phenolate (deep red) to alkyl alkoxy ester (cream colored). The reaction was definitely stepwise; two stages of water elimination occurred.

The monoalkylation of methyl 2-hydroxy-4-nitrobenzoate by means of *n*-butyl benzenesulfonate was incomplete after 300 hours; conversely, the monoalkylation of *n*-butyl 2-hydroxy-4-nitrobenzoate by methyl benzenesulfonate was complete in three hours. These rates are thus apparently determined by the differing solubilities of the two ester-phenolates in the xylene solvent.

The dialkylation of 2-hydroxy-4-nitrobenzoic acid by means of methyl benzenesulfonate required about six hours for completion, whereas the dialkylation by means of ethyl benzenesulfonate required about 18 hours. The dialkylation time then dropped rapidly as the size of the alkyl group increased; dialkylation by means of *n*-hexyl benzenesulfonate was complete in about three hours. Based on these observations it seems justifiable to conclude that steric effects occupy a subordinate position in these reactions.

The alkylation of an alkyl 2-hydroxy-4-nitrobenzoate failed with cyclohexyl and cyclopentyl *p*-toluenesulfonates. This failure was not unexpected, since the ready cleavage of *p*-toluenesulfonic acid from these secondary esters is known. Similarly, alkylation by means of trimethylene bromohydrin resulted in the elimination of hydrogen bromide from the bromohydrin, and with thenyl bromide extensive decomposition occurred. When 4-chloroquinoline was tried as an alkylating agent no reaction occurred; this negative result was shown by models to be due to steric effects.

The synthesis of the 2-alkoxy-4-nitrobenzoic acids from the corresponding 2-alkoxy-4-nitrobenzonitriles[6] by the action of nitrous acid[7] on the intermediate amides gave excellent results with

(1) Clinton, Laskowski, Salvador and Wilson, THIS JOURNAL, **73**, 3674 (1951).

(2) German Patent 522,064 (*Frdl.*, **17**, 2285 (1930)); *cf.* Brit. Appln. 12,340 (May 5, 1948) and U. S. Patent 1,317,250 (1919).

(3) (a) Walter and Fosbinder, THIS JOURNAL, **61**, 1713 (1939); (b) Vliet and Moore, U. S. Patent 2,288,334; (c) McIntyre and Sievers, *J. Pharmacol.*, **61**, 107 (1937); **63**, 369 (1938); McIntyre, *et al.*, *Nebraska State Med. J.*, **35**, No. 4 (1950).

(4) (a) Kraut, *Ann.*, **150**, 1 (1869); (b) Hale and Robinson, *Am. Chem. J.*, **39**, 680 (1908); (c) Simonsen and Rau, *J. Chem. Soc.*, **111**, 220 (1917); (d) Froelicher and Cohen, *ibid.*, **121**, 1652 (1922).

(5) Goldstein and Brochon, *Helv. Chim. Acta*, **32**, 2331 (1949).

(6) To be published in a separate communication.

(7) *Cf.* Bouveault, *Bull. soc. chim.*, [3] **9**, 370 (1893); Heyl and V. Meyer, *Ber.*, **28**, 2783 (1895).