The product was too deliquescent to make it practical to remove it from the reaction flask.

(b) **Trimethyl-(7-methyl-8-carbethoxy)-octylammonium Bromide.**—This compound was prepared in the same manner from 16.9 g. of ethyl ω-bromo-β-methylpelargonate and 39 g. of an approximately 16% solution of trimethylamine in anhydrous benzene. It also was extremely deliquescent, and after washing with 500 ml. of anhydrous ether, was used directly for the next synthesis.

Hydrazides of Trimethyl-ω-carboxyalkylammonium Bromides. (a) Hydrazide of Trimethyl-(5-methyl-6-carboxy)-hexylammonium Bromide.—A mixture of the crude trimethyl-(5-methyl-6-carbethoxy)-hexylammonium bromide and 7 g. of 85% hydrazine hydrate was heated to reflux for 15 minutes, enough alcohol was added to give a clear solution, and then refluxing was continued for an additional 2 hours. The solvent was removed under reduced pressure and the residual oil was washed with dry ether and chilled. There was obtained 2.5 g. (26%) of a white powder which melted at 118–122°.

Anal. Calcd. for $C_{11}H_{26}ON_3Br$: C, 44.59; H, 8.84. Found: C, 44.23; H, 9.16.

(b) **Hydrazide of Trimethyl-(7-methyl-8-carboxy)-octylammonium Bromide.**—The crude trimethyl-(7-methyl-8-carbethoxy)-octylammonium bromide, obtained previously, and 20 g. of 85% hydrazine hydrate were caused to react as described in the preceding experiment. The waxy appearing product was dissolved in hot absolute alcohol and precipitated by the addition of anhydrous ether. The solid was removed by filtration, dissolved in a small amount of hot absolute alcohol, clouded with petroleum ether (b.p. 60–68°) and allowed to solidify. There was obtained 5 g. (23%) of a hygroscopic, white powder, m.p. 136–139°.

Anal. Calcd. for $C_{13}H_{30}ON_3Br$: C, 48.13; H, 9.32. Found: C, 48.41; H, 9.60.

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Constituents of U. S. P. Colchicine. N-Formyltrimethylcolchicinic Acid Methyl Ether[1]

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RECEIVED MARCH 1, 1953

An earlier report from these laboratories[3] called attention to the presence of *ca.* 4% of 2-desmethylcolchicine[4] in commercial samples of U.S.P. colchicine. When a new sample[5] was subjected to chromatographic purification by the usual procedure[3,6] using chloroform–methanol (99:1) as eluant, an alkaloid (*ca.* 1.5% yield) having the properties of Šantavý's Substance B (N-formyltrimethylcolchicinic acid methyl ether)[7] was isolated; no 2-desmethylcolchicine was encountered. The new compound crystallized readily from ethyl acetate as pale yellow prisms which melted with decompositions at 260–262° (capillary). A comparison of this substance with Šantavý's Substance B is given in Table I.

The product was synthesized by formylation of trimethylcolchicinic acid methyl ether[8] using 98% formic acid in pyridine. Solvents were removed *in vacuo*, the residue was taken up in chloroform, washed with water and dried. Evaporation left a residue which crystallized readily from ethyl acetate to give pale yellow prisms, m.p. 260–262° dec. alone and when mixed with a sample isolated from U.S.P. colchicine.

TABLE I

	N-Formyltrimethylcolchicinic acid methyl ether from U.S.P. Colchicine[a]	Šantavý's Substance B	N-Formyl-iso-trimethylcolchicinic acid methyl ether
M.p., °C., dec.	260–262 (capillary)	264–267 (Kofler block)	252–253 (capillary)
$[\alpha]_D$ chloroform	−175 ± 1° c 1.01, t = 25°	−171.2° c 1.08, t = 22°	−315 ± 1° c 0.719, t = 25°
λ_{max} (log ε) (95% ethanol)	242.5 (4.48) 350.0 (4.24) (c 5.22 × 10^{-5} M)	247 (4.51) 350 (4.27)	244 (4.50) 342.5 (4.29) (c 5.6 × 10^{-5} M)

[a] Also synthesized from trimethylcolchicinic acid methyl ether.

Further confirmation of the configuration of our product was obtained by comparison with the iso-derivative prepared from iso-trimethylcolchicinic acid methyl ether[8] in the same manner. The product crystallized from ethyl acetate containing a little chloroform or methylene chloride as pale yellow prisms, m.p. 252–253° dec.; mixed m.p. with Substance B, 224–233° dec. For analysis it was dried to constant weight at 80° *in vacuo*.

Anal. Calcd. for $C_{21}H_{23}NO_6$: C, 65.44; H, 6.02. Found: C, 65.20; H, 5.94.

Comparative data are given in Table I; these are in agreement with previous findings[8,9] with respect to the properties of the iso- *vs.* the normal-forms in the colchicine and trimethylcolchicinic acid series.

Minor amounts of other alkaloids are present in the samples of U.S.P. colchicine which we have examined. Investigation of them will be continued. The biological effects of the N-formyltrimethylcolchicinic acid methyl ethers are being studied and will be reported elsewhere.

(1) This investigation was supported (in part) by a research grant from the National Cancer Institute of the National Institutes of Health, U.S.P.H.S.

(2) Research Associate.

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The Characterization and Degradation of Isotopic Acetic and Lactic Acids

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During a study of the biosynthesis of hyaluronic acid,[1] it became necessary to characterize and degrade small quantities of isotopic acetic and lactic acids. As the procedures developed may be of general interest, details are presented here.

The chemistry of benzimidazole derivatives of aliphatic acids has been described in a recent comprehensive review.[2] In contrast to the usual technique for characterization of aliphatic acids,[3–5] the present method involves the use of a large excess of the reagent, *o*-phenylenediamine, and removal

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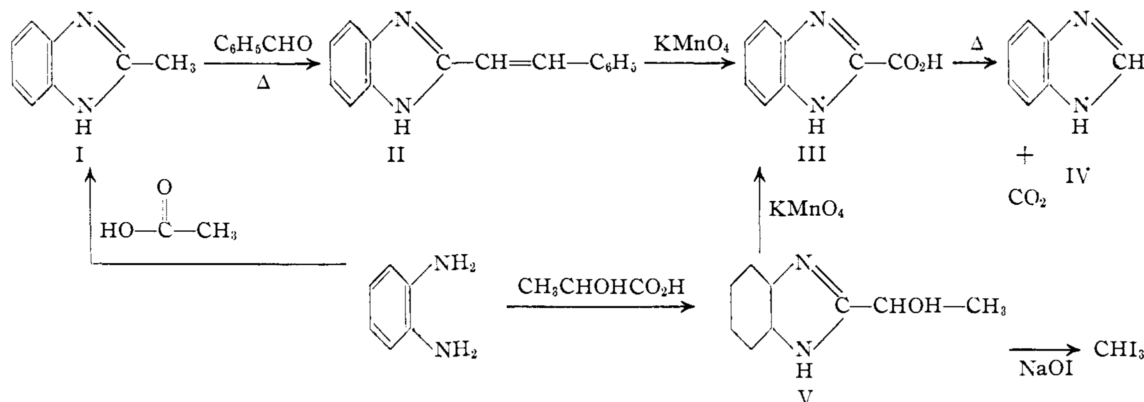
of the excess and its highly colored decomposition products by a simple procedure. This modification thus makes the benzimidazole derivatives suitable for the characterization of micro quantities of impure acetic and lactic acids.

The derivatives are employed for degradation purposes as indicated in the reaction schemes below.[6]



Experimental

Conversion of Acetic Acid to 2-Methylbenzimidazole (I).—The procedure given below is a modification of that described by Moore and Link.[7] The molar ratio of *o*-phenylenediamine dihydrochloride to acetic acid can be varied from 2:1 to 6:1 with no change in results.

Glacial acetic acid (0.400 g.), *o*-phenylenediamine dihydrochloride (4.0 g., Eastman Kodak Co.), 10 ml. of water, 1.5 ml. of 85% phosphoric acid solution and some silicon carbide boiling chips are sealed in a glass tube and the mixture is heated for 2 hours in an oven maintained at 135°. The tube is cooled, opened and placed in an oil-bath at 135° for 2 hours. The excess reagent and its degradation products are removed from the mixture by dissolving the thick sirup in water, neutralizing with solid potassium carbonate, adjusting with glacial acetic acid to *p*H 3.5–4.0 (glass electrode) and treating 2 to 4 times with benzaldehyde. Each treatment is performed by shaking the solution with 10 ml. of benzaldehyde for approximately 30 seconds followed by three extractions with chloroform (70 ml. each). Purification is considered complete when the addition of benzaldehyde yields no color. Occasionally, filtration of the mixture is necessary for complete clarification. A final extraction of the colorless solution with petroleum ether is followed by neutralization with solid potassium carbonate. Concentrated ammonium hydroxide solution (30 ml.) and 14 g. of a silver nitrate solution (4 g. of silver nitrate, 4 g. of water, 6 g. of concentrated ammonium hydroxide solution) yield a white silver salt.[8] The salt is quickly centrifuged and repeatedly washed with water. The free benzimidazole is obtained by treating a suspension of the salt in alcohol–water mixture with hydrogen sulfide, decolorizing with a *small* quantity of Norit A and concentrating the filtrate until dry. Toward the end of the concentration, 1 ml. of ammonium hydroxide solution is added. White needles (750 mg.) are obtained, m.p. 174–176° (cor.). Purification of the compound is effected either by recrystallization from dilute ammonium hydroxide solution or by vacuum sublimation at 130–145° and 10^{-5} mm. pressure. The purified product melts at 175.5–176.5°.

When micro quantities are used, the condensation is carried out as described and the benzaldehyde treatments are performed in centrifuge tubes utilizing ether rather than chloroform for the extractions. From 5.3 mg. of acetic acid, 10.0 mg. of crude 2-methylbenzimidazole (m.p. 174–176°) was obtained.[9]

Conversion of DL-Lactic Acid to Racemic 2-(α-Hydroxyethyl)-benzimidazole (V).—The procedure employed is identical with that described for acetic acid except that the 2-hour heating period in a sealed tube is omitted, the open reaction tube being inserted directly into the oil-bath. The benzimidazole can be obtained as a silver salt as described, or at the same point in the procedure it can be extracted from the neutralized aqueous solution with ether. From 0.40 g. of lactic acid, 0.40 g. of the benzimidazole (m.p. 179–180°) was obtained. Purification can be effected either by recrystallization from dibutyl ether or by sublimation *in vacuo;* m.p. 180–181°.[10]

The average yields obtained from 10 to 20-mg. samples of lactic acid were the same as indicated above.

Degradation of 2-Methylbenzimidazole.—The derivative II is obtained by heating I at 190–200° with threefold its weight of benzaldehyde in a sealed tube. After 2 hours, the oil is dissolved in an acetone–ether mixture (1:1), and an excess of concentrated sulfuric acid is added with vigorous stirring. The white sulfate salt is quickly centrifuged, washed with acetone–ether mixture, dried, and it is then heated with 4 volumes of 0.4 *N* sulfuric acid in a boiling water-bath. The mixture is allowed to cool in ice for several hours, centrifuged and washed with ice-water. The crystals are then dissolved in the minimum quantity of hot ethanol containing an excess of concentrated ammonium hydroxide solution. The addition of 3 to 4 volumes of hot water yields a colorless oil which crystallizes in the refrigerator. The compound melts at 200–201°.[11] Typical yields: from 200, 60 and 11 mg. of I—295, 65 and 13 mg., respectively of II are obtained.

To a cold solution of 60 mg. of II in 5 ml. of pyridine is added 10 mg. of potassium carbonate followed by 5 ml. of potassium permanganate solution (containing 10% excess of permanganate over that necessary for the conversion of II to III). The mixture is maintained at 0° for 2 hours and the pyridine is then removed by steam distillation. A few drops of ethanol are added and the mixture is heated to boiling and filtered with the aid of Celite. After adjusting the filtrate to *p*H 6 with acetic acid, the solution is maintained at 4° for 2 days. The desired 2-benzimidazolecarboxylic acid (III) is obtained as long, colorless needles and the yield is about one half the weight of II used in the oxidation. Additional quantities can be isolated from the mother liquors—a necessary step when the oxidation is carried out on a 10-mg. scale. The compound decomposes at 174° and is dried at room temperature for analysis as it

(6) A number of procedures have been published for the degradation of acetic and lactic acids; M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. F. Yankwich, "Isotopic Carbon," John Wiley and Sons, Inc., New York, N. Y., 1949; "Organic Reactions," Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1946, p. 307; J. Kleinberg, *Chem. Revs.*, **40**, 381 (1947); E. F. Phares, *Arch. Biochem.*, **33**, 173 (1951).

(7) S. Moore and K. P. Link, *J. Org. Chem.*, **5**, 637 (1940); *J. Biol. Chem.*, **133**, 293 (1940).

(8) If the excess reagent and its degradation products have not been completely removed by the benzaldehyde treatments, the silver salt will precipitate white but will darken rapidly. The usual precautions are observed in working with the silver nitrate-ammonium hydroxide solution.

(9) The analyses were performed by Mr. William Saschek, Department of Chemistry, University of Chicago.

(10) R. J. Dimler and K. P. Link, *J. Biol. Chem.*, **143**, 557 (1952); S. Moore, R. J. Dimler and K. P. Link, *Ind. Eng. Chem., Anal. Ed.*, **13**, 160 (1941).

(11) This substance has been obtained previously by another method: R. Weidenhagen, *Ber.*, **69**, 2270 (1936).

contains water of crystallization which is lost on more vigorous treatment.[12] Recrystallization of this compound must be performed with care.[13]

Anal. Calcd. for $C_8H_6O_2N_2 + 2H_2O$: C, 48.50; H, 5.01; N, 14.14. Found: C, 48.70; H, 5.32; N, 14.01.

For isotope experiments, III is heated to its m.p. and yields carbon dioxide and benzimidazole.[14] The carbon dioxide originates from the methyl carbon atom of the acetic acid molecule while the benzimidazole nucleus contains the carboxyl carbon atom.[15] It has been possible to perform the entire degradation starting with 11 mg. of 2-methylbenzimidazole, although in this case difficulty was experienced in isolating the pure benzimidazole.

Degradation of 2-(α-Hydroxyethyl)-benzimidazole (V).—The lactobenzimidazole isolated as described above is oxidized with potassium permanganate to III. A solution of 2.90 g. of potassium permanganate in 100 ml. of boiling water is added *all at once* to a boiling solution of 810 mg. of V and 200 mg. of sodium carbonate in 50 ml. of water. The mixture is boiled for 3 minutes and is placed on the steam-bath for 30 minutes. After the addition of small amounts of ethanol and Norit A, filtration, and adjustment to *p*H 6 with acetic acid, the colorless solution is placed in the refrigerator. After 2 days, 675 mg. of colorless needles is deposited, m.p. 174°. In other experiments, oxidation of 102 mg. and 41 mg. of V yielded 72 and 25 mg. of III, respectively. Decomposition of III as described above yields carbon dioxide (C-2 of lactic acid) and benzimidazole (contains C-1 of lactic acid).

The methyl carbon atom of lactic acid is obtained by treating V with sodium hypoiodite under the standard conditions[16] used for hydroxyethyl groups. The reaction is performed at 60° for 30 minutes and 54 mg. of V yields 22 mg. of iodoform, m.p. 117–119°. The iodoform is purified before combustion.

Isotope Experiments.—The validity of the degradation procedures was tested by degradation of benzimidazole derivatives of isotopic acetic and lactic acids. Standard techniques[6] were used to prepare 1-C^{14}-acetic acid. Group A streptococcus grown[1] in the presence of 1-C^{14}-glucose was used to produce 2-C^{14}-acetic acid and 3-C^{14}-lactic acid.[17] It is clear that in the case of the biosynthetic acids, it is possible that carbon atoms other than those indicated may contain isotope. Under these conditions, radioactivity will be obtained during the course of the degradation procedure where none is predicted. It is to be noted, however, that interpretation is always made that such results are due to the errors of the method (mixing of carbon atoms, etc.) rather than to isotope impurity of the starting material. The error of the method, as determined by these techniques, must therefore be considered the maximum error. All samples were plated as barium carbonate after combustion according to an accepted technique.[18] The activities reported have all been corrected to "infinite thickness" and are reported as counts per minute. Counting was performed using a windowless counter and "Q" gas. Table I gives the results obtained with isotopic acetic acid and Table II—with lactic acid.

The results obtained indicate that the *maximum* possible contamination of the methyl carbon atom by the carboxyl carbon atom in the case of the synthetic acetic acid is less than 0.02%, on the assumption that 1 count per minute above background is detectable. When biosynthetic acetic acid is used, and the assumption made that all of the isotope in the acid is present in the methyl carbon atom, it is possible to set an upper limit for mixing of carbon atoms during the degradation procedure at 0.37%. Calculations of this type (Table II) for the lactic acid derivatives indicate that the possible errors are of the same order of magnitude as those obtained with acetic acid. Within this range, therefore, the degradation procedure is considered valid.

TABLE I

DEGRADATION OF ISOTOPIC 2-METHYLBENZIMIDAZOLE

Starting material		$CH_3C^{14}O_2H$[a]	$C^{14}H_3C^?O_2H$[b]
Benzimidazole (C-1 of acetic acid)	c.p.m.	601	5
	× 7[c]	4207	35
CO_2 (C-2 of acetic acid), c.p.m.		0	9485
Max. possible contamination of			
C-2 by C-1	c.p.m. ratio	0–1/4207	
	%	0.02	
C-1 by C-2	c.p.m. ratio		35/9485
	%		0.37

[a] Synthetic acetate; the isolated 2-methylbenzimidazole activity was 551. [b] Bacterial product[1] after metabolism of 1-C^{14}-glucose. The isolated 2-methylbenzimidazole activity was 1,280 (× 8 = 10,240). [c] The benzimidazole activity is multiplied by 7 to correct for the dilution of C-1 of acetic acid by the carbon atoms in the benzene ring. The value 4207 compares satisfactorily with 4408 (551 × 8)—the corrected activity of the synthetic 2-methylbenzimidazole.

TABLE II

DEGRADATION OF ISOTOPIC 2-(α-HYDROXYETHYL)-BENZIMIDAZOLE

Starting material		$CH_3C^{14}HOHCO_2H$[a]	$C^{14}H_3C^?HOHC^?O_2H$[b]
Benzimidazole (C-1 of lactic acid)	c.p.m.	0	2
	× 7[c]	0	14
CO_2 (C-2 of lactic acid), c.p.m.		339	9
CHI_3 (C-3 of lactic acid), c.p.m.		0	13,200
Max. possible contamination of			
C-3 or C-1 by C-2	c.p.m. ratio	0–1/339	
	%	0.3	
C-1 or C-2 by C-3	c.p.m. ratio		14/13,200
	%		0.11

[a] Synthetic lactic acid. The activity of the lactobenzimidazole was 38 c.p.m. [b] Bacterial product[1] obtained after metabolism of 1-C^{14}-glucose. The isolated lactobenzimidazole activity was 1,437 c.p.m. (× 9 = 12,930). [c] The benzimidazole activity is multiplied by 7 to correct for dilution of C-1 of lactic acid by the carbon atoms in the benzene ring. The activity of C-2 of the synthetic lacto-benzimidazole (339) compares satisfactorily with the corrected activity of the whole molecule, 342 (38 × 9).

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Activities of Aqueous Magnesium and Barium Acetate Solutions at 25°

BY R. H. STOKES

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The measurements reported here were made in 1947 as part of a proposed study (since abandoned