phosphoric esters to thiamin, thus allowing materials containing cocarboxylase to be assayed by the thiochrome method. Such an assay gives results agreeing with biological tests.

According to our findings one International Unit is equivalent to 2.9 μg. of thiamin chloride and 4.0 μg. of cocarboxylase.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, TEMPLE UNIVERSITY]

Synthesis of Isoquinoline Acids

BY FLOYD T. TYSON

Only two of the seven possible isoquinoline acids have thus far been reported in the literature, *i. e.*, the 1-isoquinoline carboxy acid[1] and the 5- or 8-isoquinoline carboxy acid.[2] Since the derivatives of the isoquinoline acids may have useful physiological properties, it seemed desirable to proceed to synthesize members of the series not yet reported.

The 4-isoquinoline acid was prepared from the readily available 4-bromoisoquinoline[3] by reaction with cuprous cyanide to form the nitrile, followed by hydrolysis of the nitrile with concentrated hydrochloric acid.

For the preparation of the 5-, 6-, 7- and 8-isoquinoline carboxy acids by a procedure similar to that used for the 4-acid, the necessary intermediate bromoisoquinolines were prepared by the condensation of the three bromobenzalaminoacetals, using a modification of the method of Pomeranz and Fritsch.[4] The necessary acetals were prepared by the reaction of the bromobenzaldehydes with amino acetal.

The position of the carboxyl in the 5, 6, 7 and 8-acids herein described is based upon the assumption that there is no change of position of substituent groups due to intramolecular change. The substituted isoquinoline derived from *o*-bromobenzaldehyde then is 8, that derived from *p*-bromobenzaldehyde is 6, and that from *m*-bromobenzaldehyde is either 5 or 7, or a mixture of the two. A mixture of bromoisoquinolines actually is obtained from *m*-bromobenzaldehyde, as proved by the separation of derivatives of the 5- and 7-bromoisoquinoline, by fractional crystallization from aqueous dioxane of the sodium salts of the mixture of isoquinoline carboxy acids obtainable from the bromoisoquinolines.

The limiting yields obtained in the necessary reactions in each series are found in the condensation of the bromobenzalaminoacetals to the bromoisoquinolines. The yields, based upon the theoretical, for this step range from 6% in the case of the 8-bromoisoquinoline to 65% in the case of the mixture of bromoisoquinolines obtainable from *m*-bromobenzalaminoacetal.

The 5- or 8-isoquinoline acid was prepared by Jeiteles[2] from the 5- or 8-nitrile derived from the 5- or 8-isoquinoline sulfonic acid. The author has, in addition, prepared the 5- or 8-isoquinoline nitrile by the action of cuprous cyanide upon 5- or 8-bromoisoquinoline. The 5- or 8-bromoisoquinoline was obtained from 5- or 8-nitroisoquinoline as described by Claus and Hoffman.[5]

Since both the 5- and 8-acids would yield the same tricarboxybenzene,[2] by oxidation, the question of the position of the carboxyl has remained undecided. In this paper the preparation of an acid identical with Jeiteles' 5- or 8-acid from *m*-bromobenzaldehyde indicates that the acid is the 5-isoquinolinecarboxylic acid.

The methyl esters of the series of isoquinoline acids were obtained readily by the preparation of the chlorides using thionyl chloride, followed by reaction with methyl alcohol. The melting points of the methyl esters confirmed the conclusion obtained from the acids. The acid named the 5- or 8-isoquinolinecarboxylic acid,, and obtained by Jeiteles from the isoquinolinesulfonic acid,[2] is the 5-isoquinolinecarboxylic acid.

Further work is in progress in this Laboratory on the investigation of derivatives of the series of isoquinoline acids.

Experimental

Preparation of Bromobenzalaminoacetals.—Aminoacetal prepared by the procedure of Marckwald and Wohl[6] was

(1) Reissert, *Ber.*, **38**, 3429 (1905); Kaufmann, *ibid.*, **46**, 2929 (1913).

(2) Jeiteles, *Monatsh.*, **18**, 810 (1894).

(3) Edinger and Bossung, *J. prakt. Chem.*, **43**, 192 (1891).

(4) Pomeranz, *Monatsh.*, **14**, 116 (1893); **15**, 299 (1894); Fritsch, *Ber.*, **26**, 421 (1893).

(5) Claus and Hoffman, *J. prakt. Chem.*, **47**, 252 (1893).

(6) Marckwald, *Ber.*, **25**, 2355 (1892); Wohl, *ibid.*, **39**, 1953 (1906).

mixed in excess (15%) with the required amount of the desired bromobenzaldehyde, heated on the steam-bath for two hours, allowed to cool, separated from the water layer which formed, and distilled under reduced pressure.

Table I includes essential information on the preparation of the 3-isomeric bromobenzalaminoacetals.

TABLE I

Prepn.	Bromobenzaldehyde used, g.	Acetal yield, g.	B. p. of acetal °C.	Mm.	% Yield
1	50 ortho-	72	167–170	6	89
2	91 meta-	144	152–154	4	94
3	39 para-	56	160–165	4	89

Anal. Calcd. for $C_{18}H_{18}O_2NBr$: Br, 26.62. Found: for compound from prepn. 1, Br, 26.52; for prepn. 2, Br, 26.61; for prepn. 3, Br, 26.72.

Preparation of Bromoisoquinoline by Ring Closure of Bromobenzalaminoacetals.—The method of Pomeranz and Fritsch[4] was modified slightly for the preparation of the desired bromoisoquinolines. These authors recommend the use of concentrated sulfuric acid as the condensing agent. The author used a mixture of concentrated sulfuric acid and phosphoric anhydride which results in an approximately 10% increase in yield of crude bromoisoquinoline over that obtained by the use of concentrated sulfuric acid alone. The bromobenzalaminoacetal (20 g.) was added to 9 times its weight of concentrated sulfuric acid maintained at 5°.

This mixture was added with mechanical stirring, during five minutes, to a mixture of 10 g. of concentrated sulfuric acid and 20 g. of phosphoric anhydride maintained at 160°. The stirring and heating were continued for twenty-five additional minutes. The mixture was allowed to cool, ice added, and filtered. The solid residue and the filtrate were ether extracted so as to remove any neutral or acidic ether soluble material. An excess of solid sodium carbonate was added to the aqueous filtrate and the mixture steam distilled. Toward the end of the steam distillation, the solid residue was added to the alkaline liquid in the distilling vessel and the distillation continued. Thus, small amounts of bromoisoquinoline retained by the solid were recovered.

The distillate was acidified with hydrochloric acid and evaporated to dryness on the steam-bath. The hydrochloride obtained was treated with excess sodium hydroxide solution and ether extracted in a continuous extractor.

The ether extract was evaporated to remove ether, and the residue allowed to stand over calcium chloride in a vacuum desiccator and, when dry, was finally weighed as crude bromoisoquinoline. This material was used directly without further purification for the preparation of the nitrile. The mixture of crude bromoisoquinolines obtained from *m*-bromobenzalaminoacetal was an oil which solidified, on standing overnight at about 0°, to an oily solid.

The crude bromoisoquinoline obtained from *o*-bromobenzalaminoacetal and that from *p*-bromobenzalaminoacetal were both white crystalline solids. The percentage yields of crude bromoisoquinoline, compared with the theoretical, finally isolated from the condensation of *o*-, *m*-, and *p*-bromobenzalaminoacetals, were 29, 65, and 6%, respectively.

Preparation of Isoquinoline Nitriles.—The desired bromoisoquinoline was mixed thoroughly with 50% excess of cuprous cyanide. The powdered mixture was heated to 250° for forty-five minutes in a round-bottomed flask connected to a condenser. The flask was then evacuated to about 4 mm. pressure and the nitrile distilled over into the condenser tube. The nitrile could be used directly for the preparation of the isoquinoline acid. It could be recrystallized from a mixture of benzene and petroleum ether or from hot water.

The following table includes essential data on the results of typical preparations of the nitriles used.

TABLE II

Bromoisoquinoline used, g.		Nitrile yield	% Yield	M. p., °C.
10	(4-Bromo)[3]	6.5	88	104
6	(5-Bromo)[5]	3.6	81	139
11.5	(5- and 7-Bromo)[7]	7.0	82	94–115
2.0	(8-Bromo)[8]	0.8	53	133
1.1	(6-Bromo)[9]	.2	25	152

A mixed melting point determination of the 8-isoquinoline nitrile, synthesized from *o*-bromobenzaldehyde with the 5- or 8-isoquinoline nitrile of Jeiteles[2] derived from 5- or 8-nitroisoquinoline or isoquinolinesulfonic acid, resulted in a 20° depression, hence the isoquinoline nitrile of Jeiteles is probably the 5-isoquinoline nitrile.

Anal. Calcd. for $C_{10}H_6N_2$: N, 18.18. Found: for 4-isoquinoline nitrile N, 18.09; for 8-isoquinoline nitrile N, 18.22; for 6-isoquinoline nitrile N, 18.09.

Preparation of Isoquinoline Carboxy Acids.—The nitrile was mixed with approximately 9 times its weight of concentrated hydrochloric acid and heated in a closed tube at 150° for eight hours. The reaction mixture was then evaporated to dryness, redissolved in water, decolorized, if necessary, with a small amount of decolorizing carbon, filtered, and the theoretical quantity of ammonia water added to free the acid. After standing overnight the acid was filtered off and recrystallized from hot water. The yields of acid from the hydrolyses all ranged around 90% of the theoretical.

In order to select a procedure for the separation of the mixture of acids synthesized from *m*-bromobenzaldehyde, determinations were made of the solubility of the salts of 5-isoquinoline carboxy acid obtained by the method of Jeiteles,[2] and an acid obtained in small yield by the fractional crystallization from water of the mixture of acids synthesized from *m*-bromobenzaldehyde. The other component of the mixture of acids was more soluble and could not be isolated in a satisfactorily pure condition by fractional crystallization from water. The acid isolated melted at 295–297° and the 5-acid of Jeiteles melted at 280–282°. Further mixed melting point determinations gave 45° depressions. Since the acid (m. p. 295–297°) was not the 5-acid, it must be the 7-acid.

Table III includes data on the solubility of the salts of the two acids.

(7) Prepared by the series of reactions starting with *m*-bromobenzaldehyde.

(8) Prepared by the series of reactions starting with *o*-bromobenzaldehyde.

(9) Prepared by the series of reactions starting with *p*-bromobenzaldehyde.

TABLE III

SOLUBILITIES EXPRESSED AS G. SOLUTE PER 100 G. WATER

		Salts of 5-isoquinoline acid, °C.		Salts of 7-isoquinoline acid, °C.	
1	Calcium salt	0.3	32	0.2	32
		.9	100	1.8	100
2	Barium salt	2.8	29	1.5	29
		3.3	100	8.7	100
3	Magnesium salt	0.8	29	0.5	29
		3.1	100	3.6	100
	Sodium[10] salt	4.1	24	0.5	24

The mixture of acids synthesized from *m*-bromobenzaldehyde was finally separated by fractional crystallization of the sodium salts from hot aqueous dioxane (25% water and 75% dioxane by volume). By this process it was found that the mixture of acids obtained by hydrolysis of the nitriles contained about 53% of the 7-acid and 47% of the 5-acid.

The 5-isoquinolinecarboxylic acid gave no melting point depression when mixed with the 5-isoquinolinecarboxylic acid (heretofore termed 5- or 8-isoquinolinecarboxylic acid[2]) derived from the corresponding nitroisoquinoline or isoquinoline sulfonic acid. On the other hand, the 7-isoquinoline acid gave a melting point depression of 45° when mixed with the 5-acid. Further, a melting point depression of 30° was obtained with the 8-acid, derived from *o*-bromobenzaldehyde, and the 5-acid.

Table IV includes data on the melting points of the prepared acids.

TABLE IV

Isoquinoline carboxy acid	M. p., °C.
4-Carboxy	264–266
5-Carboxy	280–282
6-Carboxy	355–360 (with gradual darkening)
7-Carboxy	295–297
8-Carboxy	292–294 (with gradual darkening)

TABLE V

ANALYSES

	C, %	H, %	N, %
Anal. Calcd. for $C_{10}H_7O_2N$	69.36	4.08	8.09
Found for the 4-acid	69.20	4.06	8.10
Found for the 5-acid	69.02	3.96	8.10
Found for the 6-acid	69.25	3.94	8.15
Found for the 7-acid	68.93	4.16	8.03
Found for the 8-acid	69.05	4.09	8.03

Preparation of Methyl Esters of Isoquinolinecarboxylic Acids.—For the preparation of the required acid chloride, thionyl chloride purified by the method described by Fieser[11] was used. This purification of commercial colorless thionyl chloride was found to be essential for smooth production of the esters. Each 1 g. of acid was mixed with 10 cc. of thionyl chloride, refluxed for five minutes on a water-bath and excess thionyl chloride removed by evaporation under reduced pressure using a water pump. The residual solid, probably the hydrochloride of the acid chloride, was not purified but treated directly with 10 cc. of absolute methyl alcohol. After refluxing for five minutes on a water-bath, excess alcohol was removed by evaporation and sufficient water added to dissolve the solid at room temperature. The aqueous solution was treated with decolorizing carbon at room temperature, filtered, and an excess of an aqueous solution of potassium bicarbonate was added. The precipitated white crystalline methyl ester was filtered and dried at room temperature. The yields of the methyl esters, using 1-g. portions of the acids, were generally over 90% of the theoretical. The melting points of the methyl esters are summarized in the following table.

TABLE VI

Methyl isoquinolinecarboxylate	4	5	6	7	8
M. p., °C.	81	66	95	100	73

The melting point of the above 5-methyl ester was identical with the methyl ester of the 5-isoquinolinecarboxylic acid derived from the corresponding nitroisoquinoline or isoquinolinesulfonic acid, and gave no mixed melting point depression with it.

Anal. Calcd. for $C_{11}H_9O_2N$: N, 7.48. Found: for methyl 4-isoquinolinecarboxylate N, 7.48; methyl 5-isoquinolinecarboxylate N, 7.51; methyl 6-isoquinolinecarboxylate N, 7.53; methyl 7-isoquinolinecarboxylate N, 7.50; methyl 8-isoquinolinecarboxylate N, 7.49.

Summary

1. The ortho, meta, and para bromobenzalaminoacetals have been prepared.

2. The 6-, 7-, and 8-isoquinoline nitriles and carboxy acids have been prepared.

3. The "5- or 8-isoquinoline acid" of Jeiteles is found to be the 5-acid.

4. The condensation of *m*-bromobenzalaminoacetal has been found to form both 5- and 7-bromoisoquinoline.

5. The methyl esters of 4-, 5-, 6-, 7-, and 8-isoquinolinecarboxylic acid have been prepared.

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(10) Sodium salt extremely soluble in water; solvent used was 25% water and 75% dioxane by volume.

(11) L. F. Fieser, "Experiments in Organic Chemistry," D. C. Heath and Co., Boston, Mass., 1935, p. 339.