Anal. Calcd. for $C_{17}H_{19}BrO_2$: C, 60.90; H, 5.71; Br, 23.84. Found: C, 60.88; H, 5.92; Br, 23.82.

1-Chloro-2-benzhydryloxy-3-methoxypropane.—1,2-Epoxy-3-methoxypropane (0.68 mole) and redistilled benzhydryl chloride (0.33 mole, b.p. 97° at 0.2 mm.) were put in a 300 ml., three-neck, round bottom flask equipped with an inlet tube, thermometer, condenser, and outlet tube. The system was flushed with nitrogen, a bubble-counter attached to the outlet tube, and the flow rate adjusted to maintain a slight positive pressure. When the temperature had been raised to approximately 90°, and 0.5 ml. of water was added. The mixture was stirred under nitrogen at a temperature of 115° to 125° for 4 days. The product was cooled under nitrogen at a slightly increased flow rate. The excess epoxide was partially removed by distillation at atmospheric pressure. The residue was distilled bulb-to-bulb to give 78% yield of crude 1-chloro-2-benzhydryloxy-3-methoxypropane, b.p. 138–144° at 0.2 mm. On cooling to room temperature, the distillation residue (14%) partially solidified. The addition of methanol facilitated crystallization and subsequent filtration. The melting point and NMR spectrum indicated that the product thus obtained was dibenzhydryl ether. The crude 1-chloro-2-benzhydryloxy-3-methoxypropane was redistilled at 125–126° at 0.1 mm., through a 6-in. Vigreux column.

Anal. Calcd. for $C_{17}H_{19}ClO_2$: C, 70.21; H, 6.59; Cl, 12.19. Found: C, 70.47; H, 6.77; Cl, 12.06.

Preparation of 1-Aryl-4-(2-benzhydryloxy-3-methoxypropyl)piperazines.—*N*-Phenylpiperazine (0.1 mole) and sodium carbonate (0.1 mole) were heated with stirring for about 0.5 hr. at 100° to 135°. The mixture was cooled to approximately room temperature and 1-chloro-2-benzhydryloxy-3-methoxypropane (0.1 mole) was added dropwise. The mixture was then heated at 115–125° with stirring for several hours. The solids were removed by filtration, dissolved in aqueous alkali, and extracted with ether. The dried ether layer was combined with the filtrate and evaporated on the steam bath. The residue failed to crystallize on cooling. Additional *N*-phenylpiperazine (0.1 mole) was added and the mixture was heated on the steam bath. Needles began to form almost immediately. The heating was continued with occasional shaking for about 12 hr. The product was then thoroughly mixed with ether, filtered, and the ether evaporated on the steam bath. The excess phenylpiperazine was removed by distillation under vacuum and the residue was poured into methanol. Ether was added until the product precipitated as crystals rather than an oil when cooled. The yield was 34% and upon recrystallization from a methanol–ether solution, the product melted at 63.5–65°.

Anal. Calcd. for $C_{27}H_{32}N_2O_2$: C, 77.84; H, 7.74; N, 6.73. Found: C, 77.77; H, 7.73; N, 6.53. The monohydrochloride melted at 141°. Neut. equiv.: calcd., 453; found, 458.

1-*p*-Chlorophenyl-4-(2-benzhydryloxy-3-methoxypropyl)-piperazine was similarly prepared from *N*-*p*-chlorophenylpiperazine and 1-bromo-2-benzhydryloxy-3-methoxypropane. The yield was 70% and when recrystallized from a methanol–ether solution the product melted at 89–89.5°.

Anal. Calcd. for $C_{27}H_{31}ClN_2O_2$: C, 71.90; H, 6.93; N, 6.21. Found: C, 72.94; H, 7.00; N, 6.04.

Ether Cleavage of 1-Phenyl-4-(2-hydroxy-3-methoxypropyl)piperazine with Hydrobromic Acid.—1-Phenyl-4-(2-hydroxy-3-methoxypropyl)piperazine (0.008 mole) was dissolved in 40% aqueous hydrobromic acid (50 ml.) and heated to boiling. Solid sodium hydroxide was added until the solution was basic. The solution was extracted with ether and the extract was dried and evaporated on the steam bath. A solid product was obtained and recrystallized from benzene–heptene solution, m.p. 106.5–107.5°. If the product is assumed to be 1-phenyl-4-(2,3-dihydroxypropyl)piperazine, the yield was 53%.

Ether Cleavage of 1-Phenyl-4-(2-benzhydryloxy-3-methoxypropyl)piperazine with Hydrobromic Acid.—1-Phenyl-4-(2-benzhydryloxy-3-methoxypropyl)piperazine (0.005 mole) was treated with 40% aqueous hydrobromic acid (50 ml.) using the same procedure described immediately above. Assuming the product also to be the same, the yield was 27%, m.p. 106.5–107.5°.

Reaction of 1-Phenyl-4-(2-hydroxy-3-chloropropyl)piperazine with Aqueous Sodium Hydroxide.—An unweighed amount of 1-phenyl-4-(2-hydroxy-3-chloropropyl)piperazine was refluxed several hours in 25% aqueous sodium hydroxide. The oil layer was removed, dissolved in benzene, and extracted several times with water. The water extracts were combined and extracted once with benzene. The benzene solution was dried and the benzene removed by evaporation on the steam bath. The residue crystallized upon cooling. The crude product melted 103–105°.

A mixture of the products obtained from the above three reactions failed to show a depression in melting point. The infrared spectrum of the product was consistent with its identification as 1-phenyl-4-(2,3-dihydroxypropyl)piperazine.

Preparation of 1-Phenyl-4-(2-hydroxy-3-chloropropyl)-piperazine.—Epichlorohydrin (0.25 mole) was added slowly to an ethereal solution of *N*-phenylpiperazine (0.25 mole) at room temperature. The mixture was stirred for about 24 hr. and the solid reaction product removed by filtration; yield 77%, recrystallized from ether, m.p. 63.5–64.5°.

Anal. Calcd. for $C_{13}H_{19}ClN_2O$: C, 61.28; H, 7.51; N, 10.89. Found: C, 61.46; H, 7.40; N, 11.24.

Amino Acid Derivatives of Kojic Acid

GEORGE O'BRIEN, J. M. PATTERSON, AND J. R. MEADOW

Department of Chemistry, University of Kentucky, Lexington, Ky.

Received November 20, 1961

An extension of our study on physiologically active derivatives from kojic acid, 5-hydroxy-2-hydroxymethyl-4*H*-pyran-4-one, has led to the preparation of several new derivatives of the latter from amino acids with the substituents entering position 6 of kojic acid. Some amino acids were found to be unreactive.

The present work represents a continuation of our interest in preparing and investigating kojic acid derivatives which might have some potential physiological activity. A group of amino derivatives of kojic acid was discussed in a previous article[1] from this laboratory.

(1) G. O'Brien, J. M. Patterson, and J. R. Meadow, *J. Org. Chem.*, **25**, 86 (1960).

TABLE I
MANNICH DERIVATIVES OF KOJIC ACID WITH AMINO ACIDS

Mannich Derivative Produced from:	Formula	M.P.	Nitrogen, % Calcd.	Nitrogen, % Found	Neutralization Equivalent Calcd.	Neutralization Equivalent Found
Glycine	$C_{16}H_{17}NO_{10}$	135–136[a]	3.65	3.62	383	390
Sarcosine	$C_{10}H_{13}NO_6$	173[a,b]	5.76	5.67	243	254
Taurine	$C_{16}H_{19}NO_{11}S$	216[a,c]	3.23	3.37	...	...
DL-Valine	$C_{12}H_{17}NO_6$	216–217[a]	5.16	5.33	271	273
DL-Leucine	$C_{13}H_{19}NO_6$	225[a]	4.91	5.01	285	284
DL-Isoleucine	$C_{13}H_{19}NO_6$	204[a]	4.91	4.71	285	286
DL-Methionine	$C_{12}H_{17}NO_6S$	194–196[a]	4.62	4.67	303	303
L-Proline	$C_{12}H_{15}NO_6$	168–169[a]	5.13	5.13	269	269

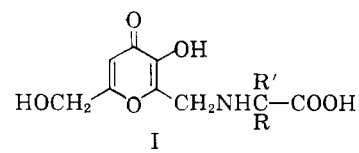
[a] Melted with decomposition. [b] Melting point after digestion in absolute methanol. [c] Melting point obtained if solid placed on block at 200°; decomposes without melting if heated slowly.

The use of amino acids as the amine component in the Mannich reaction has not been reported in the literature. In some unpublished work here,[2] it was observed that glycine and sarcosine can be used as the amine component in a Mannich reaction with 2,4,5-trichlorophenol and with 6-chlorothymol. The reaction of these amino acids with phenols involved a longer reaction time and increased difficulty of isolation of product, when compared with the Mannich reaction between these phenols and amines such as morpholine. At present, it appears that the Mannich reaction of amino acids with phenols may not be applicable to all types of phenols.

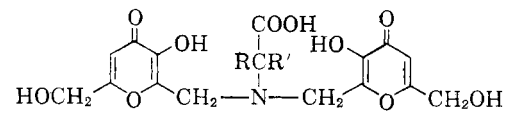
Kojic acid has been found to react with a number of amino acids of widely varying structure. Although there is considerable variation in the ease with which the Mannich derivatives may be obtained, some reactions, *viz.*, with valine, required only fifteen minutes for completion; the yield of product was good and the derivative was quite pure. Thus kojic acid again shows unusually high reactivity in the Mannich reaction when compared to a true phenol.[1]

The reaction of kojic acid with amino acids and formaldehyde is not as simple as the reaction with other types of amines. Each amino acid seems to introduce problems of its own, due to variations in structure and solubility characteristics. In general, it was possible to replace only one hydrogen atom of the amino group by a kojic acid substituent, as indicated in I below. This was true in the reaction of kojic acid with sarcosine, DL-valine, DL-leucine, DL-isoleucine, DL-methionine, and L-proline. However, in the case of glycine and taurine, Mannich derivatives of kojic acid were obtained in which both hydrogen atoms of the amino group had been replaced, as in II below. Hindrance factors no doubt play an important part in determining the structure of these amino acid derivatives of kojic acid. Details and physical properties of the Mannich compounds are given in Table I.

No Mannich derivatives could be obtained from L-aspartic acid, L-asparagine, L-glutamic acid, L-glutamine, DL-phenylalanine, and DL-tyrosine. The insolubility of the amino acid in the reaction mixture appears to be the chief factor responsible for the failure of these amino acids to react. Tyrosine and phenylalanine are virtually insoluble in aqueous alcohol unless strong acids or bases are added; prolonged heating was ineffective. Glutamic acid, although slightly soluble in the reaction mixture, failed to yield an identifiable product. It is suspected that the presence of two carboxyl groups may have had some inhibiting effect on the reaction in this case.



I



II

Glycine and sarcosine are examples of amino acids which reacted readily with kojic acid and produced products which were easily isolated from the reaction mixture when conditions were properly controlled. It was most convenient to substitute both hydrogens of the amino group in glycine, since attempts to substitute only one hydrogen atom, by limiting the amount of kojic acid, led to mixtures which could not be purified. Also, use of a significant excess of kojic acid led to a product having a high equivalent weight. This was most likely due to the formation of a kojic acid–formaldehyde condensation product, which was difficult to separate from the Mannich derivative. The reaction of sarcosine with kojic acid involved no difficulties when the amino acid was used, and the product was pure. However, use of the sodium salt of sarcosine was not at all successful. This is consistent with the observations of Alexander and Underhill[3] on the mechanism of the Mannich reaction. In addition to the difficulty of purification, it is believed that

(2) J. R. Meadow, J. E. Berger, and G. O'Brien, unpublished observations.

(3) E. R. Alexander and E. J. Underhill, *J. Am. Chem. Soc.*, **71**, 4014 (1949).

the presence of large amounts of sodium chloride may have led to serious inhibition of the reaction. Our previous experience lends support to this supposition. Valine was an ideal amino acid for the reaction with kojic acid. It was sufficiently soluble in the mixture to permit rapid and complete conversion to the Mannich derivative, while the product precipitated nicely from the reaction mixture almost as soon as formed.

Taurine did not react as readily with kojic acid as most other amino acids investigated, so that a longer reaction time was required. Since taurine is a strong acid, it might be expected that a certain amount of condensation of kojic acid with formaldehyde would take place. This could not be avoided by adjusting the *p*H of the reaction mixture; the cations, so introduced, would be difficult to isolate from the product.

Leucine and isoleucine reacted similarly with kojic acid, despite the close proximity of the methyl group (on the beta carbon adjacent to amino group) in the latter. Since there was no observed difference in behavior between leucine and isoleucine when the DL-forms were compared, it may be assumed that the orientation of a methyl group is not an important hindrance factor in the formation of Mannich derivatives of these amino acids.

The results of this work, as well as that based on our previous experience, indicate pronounced reactivity of kojic acid in the Mannich reaction which involves electrophilic substitution in position 6 of the kojic acid ring. Activation of this position is presumed to be caused by the presence of a free enolic hydroxyl group. No substituents could be found in position 3 of the ring.

Experimental

Melting point determinations were made with a Fisher-Johns apparatus and are uncorrected. Neutralization equivalents were obtained by the method of Seaman and Allen.[4] Nitrogen analyses were determined by a modification of the procedure by McKenzie and Wallace.[5] The results are summarized in Table I.

Glycine Mannich Derivative of Kojic Acid.—Attempts to substitute only one hydrogen of the amino group in glycine led to mixtures. The conditions described have been chosen to permit optimum substitution of both hydrogen atoms. Three grams (0.04 mole) of glycine was mixed with 12.0 g. (0.14 mole) of 37% aqueous formaldehyde and 50 ml. of 95% ethanol. The mixture was boiled gently for 10 min., after which 12.0 g. (0.084 mole) of kojic acid was added. The reaction mixture was then chilled for 2 or 3 hr. at −20° and filtered. The yield of faintly yellow powder, m.p. 132–134°, was 9.6 g. (62%). Recrystallization from ethanol–water (3:1) gave 7.0 g., melting at 134–136°. A sample for analysis was obtained by digesting the recrystallized material in absolute methanol, giving a white powder which melted at 135–136° with decomposition.

Anal. Calcd. for $C_{16}H_{17}NO_{10}$: N, 3.65; neut. equiv., 383. Found: N, 3.62; neut. equiv. 390. (See Table I for additional data.)

(4) W. Seaman and E. Allen, *Anal. Chem.*, **23**, 592 (1951).

(5) H. A. McKenzie and H. S. Wallace, *Australian J. Chem.*, **7**, 55 (1954).

DL-Valine Mannich Derivative of Kojic Acid.—An attempt was made to substitute both hydrogens of the amino group in valine by employing a large excess of kojic acid and formaldehyde (3 moles per mole of valine) and increasing the amount of solvent. However, only the monosubstituted valine derivative was obtained (in 80% yield). Apparently, steric effects and the low solubility of the monosubstituted derivative prevented further reaction.

Three and two-tenths grams (0.04 mole) of 37% aqueous formaldehyde was mixed with 3.5 g. (0.03 mole) of DL-valine in a mixture of 15 ml. of water and 15 ml. of 95% ethanol; this was then warmed gently until most of the valine dissolved. Five grams (0.035 mole) of kojic acid was then added in small portions over a 10-min. period. The resulting mixture was boiled gently until the valine Mannich derivative began to crystallize (usually 10 to 15 min.) after which 25 ml. of 95% ethanol was added. The solution was chilled, filtered, and the product was washed with 95% ethanol followed by ether. Six and four-tenths grams (80%) of a white powder, m.p. 215–217°, was obtained. Recrystallization from 95% ethanol sharpened the m.p. to 216–217° with decomposition. (It was difficult to recrystallize large quantities of the compound because of its low solubility in ethanol.)

Taurine Mannich Derivative of Kojic Acid.—Three and two-tenths grams (0.04 mole) of 37% aqueous formaldehyde was mixed with 1.25 g. (0.01 mole) of taurine in 60 ml. of 50% ethanol. The mixture was boiled gently until most of the taurine had dissolved, after which 3.2 g. (0.022 mole) of kojic acid was added. The boiling was continued for at least 20 min., and then the reaction mixture was allowed to evaporate at room temperature. The gummy residue, so obtained, was treated with 100 ml. of acetone, manipulating the mass with a stirring rod until crystals appeared. Recrystallization from 95% ethanol gave 2.0 g. (42%) of white powder. An analytical sample was obtained by digesting this material in absolute methanol. The Mannich derivative melted at approximately 216° with decomposition if placed on the heating block at 200° or more, but charred without melting if heated slowly over a wide temperature range. It was not possible to determine the neutralization equivalent.

L-Proline Mannich Derivative of Kojic Acid.—This heterocyclic amino acid reacted quite satisfactorily with kojic acid and formaldehyde. The product, however, was water-soluble and had to be isolated from the reaction mixture by addition of ether.

One and two-tenths grams (0.01 mole) of L-proline was dissolved in 30 ml. of 95% ethanol and 1.3 g. (0.016 mole) of 37% aqueous formaldehyde was added. The mixture was allowed to stand for 15 min. at room temperature and then 1.42 g. (0.01 mole) of kojic acid was added. The flask was shaken vigorously to dissolve the kojic acid, but was not heated. The reaction mixture was allowed to stand for 3 hr., ether was then added until the solution became turbid (about 60 ml. of ether), and the mixture was thoroughly chilled overnight. A white powder, so obtained, was recrystallized from an ethanol–ether mixture, giving 1.8 g. (70%) of a water-soluble product melting at 168–169° with decomposition.

Sarcosine Mannich Derivative of Kojic Acid.—Attempts to obtain a derivative of sarcosine by using sodium sarcosinate were unsuccessful, even when the *p*H was adjusted to 4.0 with hydrochloric acid. An increase in the ionic strength should cause an inhibiting effect on the reaction, according to views of Alexander and Underhill.[3]

However, when the free amino acid was prepared from sodium sarcosinate and used with kojic acid, no difficulty was encountered. Three and six-tenths grams (0.04 mole) of sarcosine (free amino acid) was boiled for 10 min. with 6.4 g. (0.08 mole) of 37% aqueous formaldehyde and 50 ml. of 95% ethanol. Six and two-tenths grams (0.044 mole) of kojic acid was then added and the mixture warmed gently the until latter dissolved. Since crystallization had not taken place after standing overnight, the solution was allowed to evaporate at room temperature in an evaporating dish.

The gummy residue became crystalline after trituration with 200 ml. of acetone, and the white solid was recrystallized from 95% ethanol. Four and eight-tenths grams (50%) of a product softening at 134–136° was obtained. Digestion of 1 g. of this material in 40 ml. of absolute methanol produced 0.9 g. of white powder, melting at 173° with decomposition.

Other Amino Acid Derivatives of Kojic Acid.—The conditions used for the preparation of DL-leucine and DL-isoleucine Mannich derivatives of kojic acid were similar to those used for the valine derivative. The methionine derivative was prepared in a similar manner, although more heating was required.

Attempted Preparation of Derivatives from Other Amino Acids.—L-Aspartic acid, L-asparagine, L-glutamic acid, L-glutamine, DL-phenylalanine, and DL-tyrosine failed to give satisfactory results with kojic acid and formaldehyde. Phenylalanine and tyrosine were too insoluble to permit reaction. Glutamic acid, glutamine, and asparagine appeared to be too unreactive. These amino acids were recovered more or less unchanged when conditions similar to the preparation of the valine derivative were used. If prolonged heating was used, mixtures, contaminated with decomposition products, were obtained.

Acknowledgment.—The authors wish to thank the Geschickter Fund for Medical Research, Washington, D. C., for help and cooperation in carrying out this research program.

Antineoplastic Agents. VI. Mannich Base Nitrogen Mustards (Part B)[1-3]

GEORGE R. PETTIT AND JOSEPH A. SETTEPANI

Department of Chemistry, University of Maine, Orono, Me.

Received December 26, 1961

A Mannich-type reaction involving bis(2-chloroethyl)amine, formaldehyde, and several amides has been investigated. Bis(2-chloroethyl)amine was shown to condense in ethanol–formalin solution with phthalimide, saccharin, isatin, and isatin thiosemicarbazone to yield (51–89%) the corresponding nitrogen mustard Mannich bases II, IV, and VI. The reaction was found to follow other courses under acidic conditions. Formation of *N*-methylbis(2-chloroethyl)amine and conversion of saccharin to alkoxymethylene (VII) and bismethylene (VIII) derivatives occurred in acidic media.

Nitrogen mustards, *viz.*, *N*-substituted bis(2-chloroethyl)amines, are commonly prepared[4] by chlorinating an appropriate bis(2-hydroxyethyl)-amine using thionyl chloride or a phosphorus halide.[4a-e] Other procedures include: condensations employing bis(2-chloroethyl)amine in a Mannich-type reaction [1,4f] or with an alkyl halide,[4g] aluminum chloride–lithium aluminum hydride reduction of *N*,*N*-bis(2-chloroethyl)amides,[2] and syntheses employing a previously prepared nitrogen mustard.[4h-l]

Recently we described the preparation of nitrogen mustards derived from cyclohexanone and a variety of acetophenones employing a Mannich reaction between the respective ketone, formaldehyde, and bis(2-chloroethyl)amine.[1] The potential value of this mild reaction as a route to nitrogen mustard derivatives of certain natural products emphasized the desirability of extending our initial investigation to Mannich-type reactions involving *N*-substitution (*e.g.*, I→II).

A number of amides and nitrogen heterocyclic compounds containing a labile N—H bond have been condensed with formaldehyde and a primary or secondary amine.[5] Generally, a neutral solvent composed of ethanol and formalin provides a satisfactory medium for this reaction. On occasion condensation is essentially complete within a few minutes at ice bath temperature[6]; however, reaction periods of one or more hours at room temperature or above are more frequently encountered.[5]

In contrast to our previous experience[1] with bis-(2-chloroethyl)amine in Mannich reactions leading to C-alkylation, this amine readily condensed with phthalimide (Ia), saccharin (III), isatin (Va), and isatin thiosemicarbazone (Vb) in ethanol–formalin (37%) solution. The corresponding Mannich base nitrogen mustards (II, IV and VI) were obtained in

(1) Part A; G. R. Pettit and J. A. Settepani, *J. Med. Pharm. Chem.*, **6**, 296 (1962).

(2) Refer to G. R. Pettit, M. F. Baumann, and K. N. Rangammal, *J. Med. Pharm. Chem.*, **6**, in press (1962) for contribution V.

(3) This investigation was aided by Grant No. T-79B from the American Cancer Society.

(4) For recent examples, consult ref. 2 (footnote 4), and: (a) E. J. Reist, R. R. Spencer, M. E. Wain, I. G. Junga, L. Goodman, and B. R. Baker, *J. Org. Chem.*, **26**, 2821 (1961); (b) A. H. Soloway and E. Nyilas, *ibid.*, **26**, 1091 (1961); (c) S. Chu, J. E. Harris, and H. G. Mautner, *ibid.*, **25**, 1759 (1960); (d) J. F. Allen and N. B. Chapman, *J. Chem. Soc.*, 1482 (1960); (e) T. L. Fletcher and W. H. Wetzel, *J. Org. Chem.*, **25**, 1348 (1960); (f) R. C. Elderfield and J. R. Wood, *ibid.*, **26**, 3042 (1961); (g) Ya. L. Gol'dfarb and M. S. Kondakova, *Zhur. Obshchei Khim.*, **30**, 102 (1960). (h) F. D. Popp, *J. Org. Chem.*, **26**, 3020 (1961); (i) A. M. Creighton, L. N. Owen, and G. R. White, *J. Chem. Soc.*, 2375 (1961); (j) R. H. Wiley and G. Irick, *J. Org. Chem.*, **26**, 593 (1961); (k) F. Bergel and J. A. Stock, *J. Chem. Soc.*, 3658 (1960); (l) A. Ya. Berlin and V. P. Bronovitskaya, *Zhur. Obshchei Khim.*, **30**, 324 (1960).

(5) A review of recent studies pertinent to this subject and leading references to prior investigations may be obtained by consulting: V. I. Stravrovskaya and M. O. Kolosova, *Zhur. Obshchei Khim.*, **30**, 689 (1960); J. H. Burckhalter and D. R. Dill, *J. Org. Chem.*, **24**, 562 (1959); W. J. Gottstein, W. F. Minor, and L. C. Cheney, *J. Am. Chem. Soc.*, **81**, 1198 (1959); S. Swaminathan, S. Ranganathan, and S. Sulochana, *J. Org. Chem.*, **23**, 707 (1958); H. W. Heine, M. B. Winstead, and R. P. Blair, *J. Am. Chem. Soc.*, **78**, 672 (1956); and a text by B. Reichert, *Die Mannich-Reaktion*, Springer Verlag, Berlin, 1959.

(6) *Cf.*, C. C. Bombardieri and A. Taurins, *Can. J. Chem.*, **33**, 923 (1955).