trated to a cream colored crystalline product; yield 0.42 g., m.p. 153–159°. Recrystallization from acetone gave colorless crystals of dihydrotigogenin melting at 165–166°, $[\alpha]_D^{16}$ 0.0°.[5,12]

The diacetate (acetic anhydride–pyridine, 1 hr. at 90–95°) melted at 115° and was identical (mixed melting point and infrared comparison) with an authentic specimen (m.p. 116–117°) of dihydrotigogenin diacetate (IIe).[5,17]

Desoxytigogenin (Id). In one experiment, a solution of tigogenone (Ig, 0.14 g.)[18] in ethanedithiol (3 ml.) was treated with 1 drop of 70–72% perchloric acid. Colorless crystals began to separate within several minutes. However, the reaction was allowed to proceed for 3 hr. at 25° before dilution with ether and 2*N* sodium hydroxide. The ethereal solution was washed successively with 2*N* sodium hydroxide and water before removing the dry (sodium sulfate) solvent *in vacuo*. Chromatography of the pale yellow colored crystalline residue, in 4:1 petroleum ether–benzene on 10 g. of Merck activated alumina, followed by elution with the same solvent, gave 0.15 g. of colorless product melting at 299–302°. Two recrystallizations from chloroform–ethyl acetate afforded a pure sample of tigogenin 3-ethylenethioketal (Ic) as needles, m.p. 307–309°,[19] $[\alpha]_D^{16}$ −67.7°.

Anal. Calcd. for $C_{29}H_{46}O_2S_2$: C, 70.97; H, 9.44; S, 13.07; mol. wt. 490.8. Found: C, 70.62; H, 9.70; S, 13.15; mol. wt. (Rast), 465.

Desulfurization of the ethylenethioketal Ic (0.027 g.) with W-4 Raney nickel[20] (0.5 ml.) was carried out in ethanol (30 ml.) employing a 4-hr. reflux period. The solvent was removed after filtering the hot reaction mixture through Celite. The crystalline residue recrystallized from ethanol as colorless plates (0.011 g.), m.p. 174–175°. Mixed melting point determination with an authentic sample (m.p. 174–175°) of desoxytigogenin (Id)[18] was undepressed.

Dihydroxydesoxytigogenin (IId). Conversion of desoxytigogenin (Id, 0.72 g.) to the *dihydro* derivative IId was readily accomplished. The crude oily product was chromatographed in petroleum ether on 10 g. of Merck acid washed alumina. Elution with petroleum ether–benzene (1:1) afforded 0.70 g. of pale yellow oil which crystallized upon trituration with acetone. Recrystallization from acetone gave colorless needles, m.p. 92–93°, $[\alpha]_D^{16}$ 0.0°.[12]

Dihydrodiosgenin (IVa). Reduction of diosgenin (IIIa, (0.50 g.) led to dihydrodiosgenin (0.45 g.). A sample recrystallized from acetone as colorless needles, m.p. 167–168°, $[\alpha]_D^{16}$ −28.9° (*cf.* ref. 5). The product gave a straw coloration with tetranitromethane. A mixed melting point with dihydrotigogenin (IIb) was 160–164°, while comparison infrared spectra (potassium bromide) were distinctly different.

Diosgenin acetate (IIIb, 0.50 g.) afforded 0.45 g. of dihydrodiosgenin melting at 163–165°. Recrystallization from acetone gave colorless needles, m.p. 165–167°.

Dihydromarkogenin (IIe). A 0.28 g. sample of markogenin[17] provided 0.25 g. of dihydromarkogenin which crystallized as colorless needles from acetone, m.p. 179–180°, $[\alpha]_D^{16}$ 0.0°.

Anal. Calcd. for $C_{27}H_{46}O_4$: C, 74.61; H, 10.67. Found C, 74.58; H, 10.61.

11α-Hydroxydihydrotigogenin (IIf). Almost quantitative conversion of 11α-hydroxytigogenin (If, 0.15 g.)[21] to the dihydro compound (IIf) was realized; yield 0.14 g. Recrystallization from acetone gave colorless rosettes of needles, m.p. 176–177°, $[\alpha]_D^{16}$ −13.0°.

Anal. Calcd. for $C_{27}H_{46}O_4$: C, 74.61; H, 10.67. Found: C, 74.74; H, 10.71.

Dihydrotomatidine (VI). Lithium aluminum hydride–aluminum chloride reduction of tomatidine (V, 0.50 g.) was carried out as previously described (*cf.* IIb). In this case, the reaction mixture was diluted with sodium hydroxide solution. Ether extraction and recovery of product in the usual manner afforded 0.35 g. of cream colored solid, m.p. 226–228°. Recrystallization from methanol-water gave colorless plates (0.20 g.) melting at 229–230°. Concentration of the mother liquors afforded the lower melting isomer (0.09 g.) as cream colored crystals, m.p. 193–195°. The isomer melting at 229–230° was found to be identical (mixture melting point determination and infrared comparison) with an authentic sample of the higher melting (230–233°) isomer of dihydrotomatidine.[13]

3β-Acetoxy-5-furostene 26-ethylenethioketal (IVb). A mixture of diosgenin acetate (IIIa, 0.5 g.), ethanedithiol (1 g., 0.9 ml.) and aluminum chloride (2 g.) in ether (5 ml.) was allowed to stand at room temperature over a 3-hr. period. Following dilution with benzene, the reaction mixture was washed successively with 2*N* sodium hydroxide and water. The residue obtained after removing the dry (sodium sulfate) solvent *in vacuo* was chromatographed in 1:1 petroleum ether–benzene on Merck activated alumina. Elution with the same solvent gave an oil which crystallized as needles from acetone; yield 0.38 g., m.p. 140°. The product was identified by mixed melting point determination and infrared comparison with an authentic sample of 3β-acetoxy-5-furostene 26-ethylenethioketal (m.p. 140–142°).[8]

ORONO, ME.

(17) This sample was kindly provided by Dr. M. E. Wall.

(18) M. E. Wall and S. Serota, *J. Am. Chem. Soc.*, **78**, 1747 (1956).

(19) Capillary tube melting point.

(20) A. A. Pavlic and H. Adkins, *J. Am. Chem. Soc.*, 68, 1471 (1946).

(21) A sample of this compound was generously provided by Dr. Carl Djerassi.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF KENTUCKY]

Amino Derivatives of Kojic Acid

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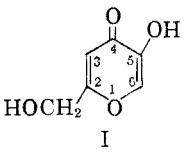
An investigation of the physiological properties of new compounds from kojic acid, 5-hydroxy-2-hydroxymethyl-4*H*-pyran-4-one has led to the synthesis of several amino derivatives which have not previously been reported. Secondary as well as tertiary aminomethyl groups were introduced into position 6 of kojic acid by use of the Mannich reaction. Factors which influence this reaction, as it applied to kojic acid, were also studied.

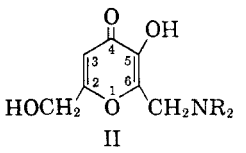
Our work on kojic acid derivatives is an extension of the program here to prepare a large number of aminophenols and aminobisphenols of various types, with the ultimate view of evaluating them as

potential chemotherapeutic agents.[1-3] Some interesting physiological properties of kojic acid have already been observed, including a definite, but weak, bacteriostatic activity, a synergistic activity when used with insecticides, cardiotoxic and cardiotonic activity, and the ability to kill human leucocytes *in vitro*.[4] As an enzyme inhibitor kojic acid has been found to decrease the activity of D amino acid oxidase in rate bearing growing transplanted tumors.[5] We would expect that the physiological properties of kojic acid should be considerably modified by the introduction of amino groups.

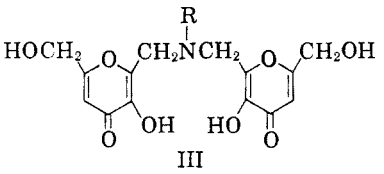
Because of its phenol-like properties kojic acid (I) should provide at least one hydrogen atom in the 6 position which can be replaced by Mannich[6] groups such as $-CH_2NHR$ and $-CH_2NR_2$ (II). The only previously reported compounds of this general type were those of Woods[7] and also Barchielli.[8] The latter prepared two derivatives which are structurally somewhat similar to Mannich bases by reaction of kojic acid with Schiff bases



I



II



III

such as benzalaniline and *p*-methylbenzalaniline. Woods[7] has reported four di-Mannich derivatives obtained in an acid medium from kojic acid, formaldehyde, and aromatic amines. He suggests that two Mannich groups entered positions 3 and 6 of the kojic acid ring in each of his compounds, although the molar ratio of reactants used in each case (1:1) would seem to favor a mono-Mannich derivative instead of a di-Mannich product. Inasmuch as there has been some difficulty in separating and obtaining pure products from the reaction mixture by the use of Woods' method, we are excluding the group of aromatic primary amines from this discussion and they will be reported later when sufficient data are available.

The present group of amine derivatives of kojic acid (II and III) was obtained by reaction of kojic acid with formaldehyde and strongly basic amines at room temperature. The reaction proceeded very rapidly and the derivatives could be isolated from the reaction mixture in good yield within thirty minutes. Using primary and secondary aliphatic amines, as well as some heterocyclic secondary amines, it was found that only one position of the kojic acid ring (position No. 6) was substituted by a Mannich group ($-CH_2NR_2$), even when an excess of amine and formaldehyde was employed (see Table 1). In the case of the primary amines tried, both hydrogens attached to the nitrogen were replaced by kojic acid rings (at the 6 position in the latter, see formula III above). For secondary amines, products corresponding to the structure in II above were obtained.

The method of Meadow and Reid[9] and also that used by Woods[10] led to the formation of resinous materials of indefinite composition when kojic acid was heated with formaldehyde and strongly basic amines such as dimethylamine, morpholine, or piperidine. In view of the sensitivity of the pyranone ring to basic reagents[11] it is understandable that the Mannich reaction involving kojic acid and amines more basic than the aromatic amines has not been described. Although ring cleavage, by hydroxide ion is reversed by the action of acids, such ring cleavage in the presence of amines could be expected to lead to any of several side reaction products, such as open chain amine derivatives, pyridones and polymers. Thus kojic acid although capable of entering into some of the substitution reactions typical of a phenol, is not as stable under these conditions as a true phenol. The success of this study depends on the possibility of kojic acid taking part in the Mannich reaction under conditions which will not produce extensive ring cleavage. The surprising observation that kojic acid reacts rapidly with morpholine and other strongly basic amines in the presence of formaldehyde at room temperature led us to the preparation and study of a large number of other similar derivatives using such exceedingly mild conditions.

We were able to isolate only products which contained a single Mannich group in the 6 position. Attempts to force a second group presumably in the 3 position failed. The supposition or conclusion that the Mannich group entered the 6 position of kojic acid is based on the following reasons: First in the nuclear substitution derivatives at present reported in the literature[12] the entering group is believed to enter the 6 position, *ortho* to the enolic hydroxyl group although no very definite

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(4) A. Beelik, *Advances in Carbohydrate Chemistry*, **11**, 145 (1956).

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(12) A. Beelik, *Advances in Carbohydrate Chemistry*, **11**, 145 (1956).

TABLE I

MANNICH DERIVATIVES OF KOJIC ACID WITH ALIPHATIC AND HETEROCYCLIC AMINES

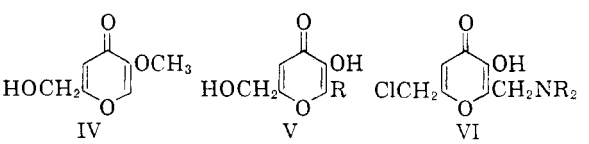
Mannich Derivative (Amine Used)	Formula	Melting Point, °C[a]	Nitrogen Analysis, %		Neutralization Equivalent	
			Calcd.	Found	Calcd.	Found
Dimethylamine[b]	$C_9H_{13}NO_4HCl$	185–186	5.94	6.06		
Diethylamine[b]	$C_{11}H_{17}NO_4HCl$	157–158	5.31	5.23		
Laurylamine	$C_{26}H_{39}NO_3$	61	2.90	2.89	484	490
Stearylamine	$C_{32}H_{51}NO_3$	72–73	2.42	2.44	578	585
Pyrrolidine[b]	$C_{11}H_{15}NO_4HCl$	168–169	5.35	5.34		
Morpholine	$C_{11}H_{15}NO_5$	156	5.81	5.70	241	241
Piperidine	$C_{12}H_{17}NO_4$	166–168	5.85	5.80	239	239
N-Methylpiperazine	$C_{12}H_{18}N_2O_4$	192	11.02	10.61	127	129
1,2,3,4-Tetrahydroquinoline	$C_{16}H_{17}NO_4$	138–139	4.87	4.74	287	280

[a] Some decomposition was apparent when all derivatives were melted on the heating block. [b] Isolated as the hydrochloride salt, washed with acetone, and recrystallized from alcohol.

evidence for this orientation has yet been presented; and secondly some indirect evidence based on our experimental work here.

Alexander and Underhill[13] have shown that an electrophilic substitution is involved in the preparation of Mannich derivatives of ethylmalonic acid. It is probable that an electrophilic substitution reaction is also involved in the preparation of Mannich derivatives of phenols and possibly those of kojic acid. The failure of 6-hydroxymethylkojic acid to enter into the Mannich reaction indicates that 6-hydroxymethylkojic acid is not an intermediate in the reaction and lends support to the idea that the Mannich reaction with kojic acid involves electrophilic substitution of the kojic acid ring. Of the two open nuclear positions of kojic acid, positions 3 and 6, only position 6 is under the activating influence of the enolic hydroxyl group of kojic acid, and only position 6 would be deactivated by the conversion of the hydroxyl group to an ether group. Position 3 is *meta* to the hydroxyl group and should be only slightly affected. With this in mind, we have carried out the following experiments.

Methyl kojate (IV) failed to undergo the Mannich reaction using both acidic and basic conditions. A similar situation exists in the phenol series; anisole has not been converted into a Mannich derivative.



The other open position of kojic acid the 3 position, is *meta* with respect to the enolic hydroxyl group, but *ortho* to a keto group. Any activity of the 3 position should arise as a result of the influence of this keto group. Since the keto group should be unaffected by the formation of methyl kojate, it is unlikely that the activity of the 3 position would be altered by forming methyl kojate. The observed lack of reactivity of methyl kojate can be explained only if it is assumed that the 6 position is the reactive position of kojic acid. Second, four mono-substituted nuclear derivatives of kojic acid (V) failed to form a Mannich derivative. The derivatives in question (V) were 6-bromokojic acid, 6-bromochlorokojic acid or 2-chloromethyl-5-hydroxy-6-bromo-4*H*-pyran-4-one, 6-hydroxymethylkojic acid, and 6,6′-methylene-bis-kojic acid. These compounds have been previously reported in the literature, and are presumed to be derivatives in which the 6 position of kojic acid has been substituted. The failure of these four mono-substituted derivatives of kojic acid to yield Mannich derivatives, may be considered an indication that the position ordinarily substituted in the Mannich reaction is blocked in these derivatives. It may be concluded that the position on the kojic acid molecule which is substituted in other nuclear substitution reactions is also substituted in the Mannich reaction.

Chlorokojic acid or 2-chloromethyl-5-hydroxy-4-*H*-pyran-4-one which contains a chloromethyl group instead of hydroxymethyl group in the 2 position reacted in the usual way to form a mono-Mannich derivative (VI). Both the 3 and 6 positions remain unsubstituted in chlorokojic acid, and the enolic hydroxyl is free. It was anticipated that alkylation of the amine by the chloromethyl group might produce a serious side reaction. However, the reactivity of the chlorine atom of chlorokojic acid was sufficiently low to permit the formation of Mannich derivatives (VI) in the usual way with several amines tried. Additional Mannich compounds are listed in Table II.

The results of this work indicate very strongly that the pronounced reactivity of kojic acid in the Mannich reaction is due to the presence of a free enolic hydroxyl group. The enhanced reactivity

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

TABLE II

MANNICH DERIVATIVES FROM SUBSTITUTED KOJIC ACID COMPOUNDS

Substituted Kojic Acid Used as Starting Material	Mannich Group Introduced in Position No. 6	Formula	Melting Point, °C.	Nitrogen Analysis, %		Neutralization Equivalent	
				Calcd.	Found	Calcd.	Found
Chlorokojic acid[a]	Morpholinomethyl	$C_{11}H_{14}ClNO_4$	140–141	5.39	5.44	260	258
Chlorokojic acid[a]	Piperidinomethyl	$C_{12}H_{16}ClNO_3$	138–139	5.43	5.41	258	257
7-*O*-Acetylkojic acid[b]	Morpholinomethyl	$C_{13}H_{17}NO_6$	120–121	4.96	5.03	283	285

[a] Prepared by reaction of kojic acid with thionyl chloride in $CHCl_3$ by a modification of the method described by Kipnis, Soloway, and Ornfelt.[22] [b] Obtained by aminolysis of 2,7-di-*O*-acetylkojic acid. The latter was prepared by the method of Maurer.[23]

of kojic acid compared to phenol is probably due to the larger anion concentration of kojic acid since kojic acid is somewhat more acidic than phenol. In addition, it appears that only one position in the kojic acid ring is sensitive to substitution by a so-called Mannich group.

EXPERIMENTAL

All melting points were taken with a Fisher-Johns apparatus, and are uncorrected. Neutralization equivalents were determined with standard perchloric acid in glacial acetic acid solution according to the method of Seaman and Allen.[14] The Kjeldahl analyses for nitrogen were determined by a modification of the method of McKenzie and Wallace,[15] with 0.025*N* sulfamic acid as the titrant. Results for the derivatives are summarized in Table I. A typical preparation procedure may be described as follows.

Morpholinomethyl derivative of kojic acid. Three and one-half grams (0.04 mol.) of freshly distilled morpholine was mixed with 3.2 g. (0.04 mol.) of 37% aqueous formaldehyde and 40 ml. of 95% ethyl alcohol. The mixture was allowed to stand 15 min., after which 4.3 g. (0.03 mol.) of kojic acid was added. The flask was stoppered and shaken vigorously for 15 min., during which time a tan crystalline solid began to form. After standing 1 hr. at room temperature the reaction mixture was chilled and then filtered. Washing with two small portions (15 ml. each) of cold 95% ethyl alcohol gave about 6.5 g. (88% yield) of nearly white leaflets, melting at 156° with decomposition. In this case recrystallization from hot alcohol failed to change the melting point; furthermore, it was observed that prolonged heating to dissolve the compound caused some decomposition to take place which resulted in a slight loss of recovered product. This and other Mannich derivatives of kojic acid were appreciably soluble in cold water.

Anal. Calcd. for $C_{11}H_{15}NO_5$: C, 54.76; H, 6.27. Found: C, 55.06; H, 6.00. (See Table I for additional analytical data.)

Piperidinomethyl derivative of chlorokojic acid. Chlorokojic acid, m.p. 165–166° (see note a, Table II) reacted rapidly with both morpholine and piperidine in a manner similar to kojic acid. Two and six-tenths grams (0.03 mol.) of freshly distilled piperidine and 3.0 g. (0.036 mol.) of 37% aqueous formaldehyde were mixed with 40 ml. of 95% ethanol and allowed to stand for about 15 min. Chlorokojic acid, 4.8 g. (0.03 mol.), was then added slowly to the mixture and shaking or stirring was continued for another 15 min. After standing at room temperature for at least 1 hr., the product was then chilled until crystallization was complete and filtered. The crystals were washed two or three times with small portions of cold 95% ethanol, then with 20 ml. of ether, after which the product was dried rapidly by suction. A yield of 6.4 g. (82% of theory) of faintly pink, crystalline powder, m.p. 138–139° (dec.), was obtained. (Analytical data are found in Table II.) It was possible to recrystallize the product from 95% ethanol, but the loss due to decomposition was high (as much as 50 to 60% when the alcohol was heated to boiling for any length of time).

Methyl kojate and the Mannich reaction. Methyl kojate (IV) was prepared by the method of Campbell, Ackerman, and Campbell.[16] It failed to form a Mannich derivative with morpholine under basic conditions used previously. Also, it failed to react under acidic conditions with hydrochloric acid present. Other substituted kojic acid compounds which failed to undergo the Mannich reaction were the following: 6-bromokojic acid, prepared by a modification of Yabuta's method[17]; 6-bromochlorokojic acid, obtained by bromination of chlorokojic acid[18] with *N*-bromosuccinimide according to Woods[19]; 6-hydroxymethylkojic acid, prepared by the method of Woods[20]; and 6,6′-methylene-bis-kojic acid, obtained by the method of Barham and Reed.[21]

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