number of mice which lost the reflex over 20 s.

Rotating Rod Test. Groups of six mice were injected intraperitoneally with the test compounds. After 30 min the mice were placed for 1 min on a rotating rod (3.5 cm in diameter, 14 rpm). The ED_{50} value was estimated from the number of mice which fell off the rod twice during the test.

Maximal Electroshock Test. Groups of six mice were injected intraperitoneally with the test compounds. After 30 min the alternating current of 25 mA was delivered for 0.15 s through corneal electrodes. The ED_{50} value was estimated from the number of mice which were protected against the tonic extensor component of the hind limbs.

Pentylenetetrazole Convulsions. Groups of six mice were injected intraperitoneally with the test compounds. After 30 min pentylenetetrazole was injected subcutaneously at a dose of 125 mg/kg. The ED_{50} value was estimated from the number of mice which were protected against death due to tonic extensive convulsions within 1 h after administration of the convulsant.

Acknowledgment. The authors thank Drs. I. Chibata, M. Miyoshi, Y. Kowa, and A. Kiyomoto for their encouragement throughout the present study.

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(9) The direct comparison of **24** with its parent compound in the CNS depressant activity was not made. However, the higher activity of **24** is supportedly shown by the values reported in the ref 2a.

(10) T. Ochiai, Y. Kudo, and R. Ishida, unpublished data.

Kojic Amine—a Novel γ-Aminobutyric Acid Analogue

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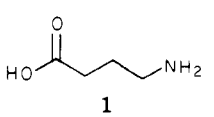
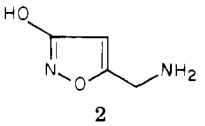
A series of compounds containing the 3-hydroxy-4*H*-pyran-4-one nucleus has been synthesized and tested as potential skeletal muscle relaxants. Reduction of 2-(azidomethyl)-5-hydroxy-4*H*-pyran-4-one (**4**) with HBr in HOAc–phenol yielded 2-(aminomethyl)-5-hydroxy-4*H*-pyran-4-one (kojic amine, **3**) in 81% yield. Reaction of 2-[(tosyloxy)-methyl]-5-(benzyloxy)-4*H*-pyran-4-one (**5**) with NH_3 gave a 40% yield of the *O*-benzyl ether of kojic amine, which was N-acylated with a series of carbobenzyloxy-protected amino acids. Complete deprotection with HBr–HOAc gave the following amino acid amides of kojic amine: glycyl (**23**), α-alanyl (**24**), β-alanyl (**25**), γ-aminobutyryl (**26**), and glycylglycyl (**27**). Among the analogues of kojic amine prepared was a series of one-carbon homologues: 2-[(methylamino)methyl]-5-hydroxy-4*H*-pyran-4-one (**7a**), 2-(1-aminoethyl)-5-hydroxy-4*H*-pyran-4-one (**8**), 6-(aminomethyl)-3-hydroxy-2-methyl-4*H*-pyran-4-one (**12**), and 2-(2-aminoethyl)-5-hydroxy-4*H*-pyran-4-one (**16**). Kojic amine (**3**) has been found to possess certain of the properties to be expected in a γ-aminobutyric acid mimetic agent, notably skeletal muscle relaxant activity. In the chronic spinal cat preparation, ED_{70} values for reduction of flexor spasms of 2.2 and 4.0 mg/kg by iv and po routes of administration, respectively, were observed for kojic amine, which was the most potent of the various hydroxypyrone derivatives investigated.

γ-Aminobutyric acid (GABA, **1**) is believed to play a



1



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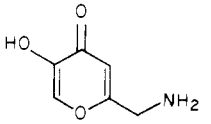

3

major role in vertebrates as an inhibitory neurotransmitter, both at the brain and spinal levels.[1,2] As GABA itself does not cross the blood–brain barrier, there is considerable interest in the development of systemically active GABA-mimetic agents. These agents might have therapeutic utility in neurological disorders such as Huntingdon's chorea,[3] schizophrenia,[4] and epilepsy,[5] as well as in analgesia[6] and in the treatment of skeletal muscle spasticity. Muscimol (**2**), a potent, orally active, naturally occurring GABA-like agent, is toxic and has been reported to cause hallucinations in man.[7] The very recent report[8] that muscimol is a potent blood pressure lowering agent when administered intracerebroventricularly indicates a possible involvement of GABA receptors in the central regulation of blood pressure. The structure of muscimol, which corresponds to the extended conformation of

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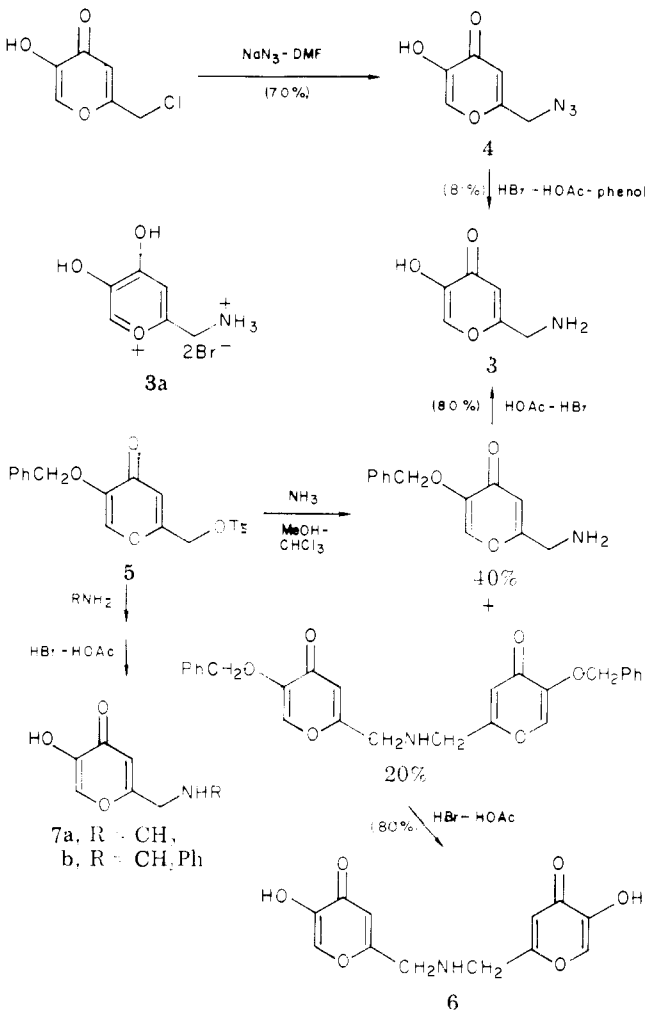
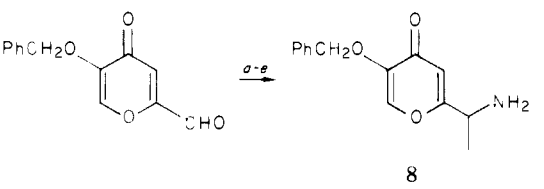
Scheme I



Scheme II



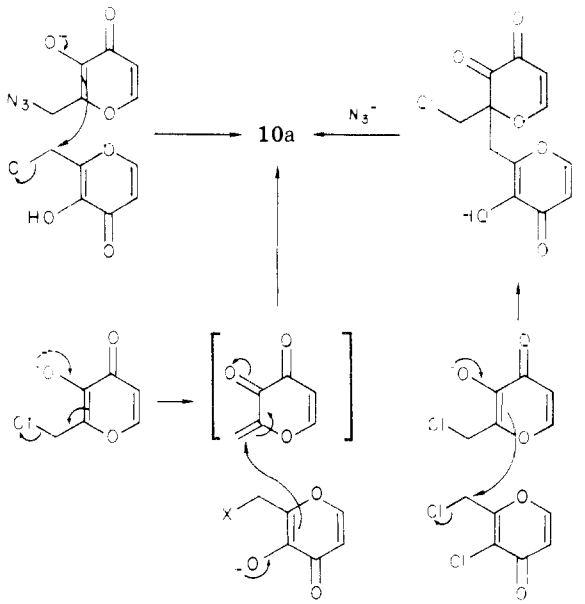
[a] MeMgI (15%). [b] $Na_2Cr_2O_7$ (80%). [c] NH_4OH (59%). [d] Zn–HOAc (100%). [e] 48% HBr (42%).

GABA,[9] suggested to us that analogous primary amines incorporating other types of cyclic carboxyl equivalents might also display GABA-mimetic actions. The pK_a values for GABA are 4.04 and 10.71,[10] while the corresponding values for muscimol are 4.78 and 8.43.[10] The reduced basicity and acidity of muscimol presumably contribute to its effective penetration into the CNS. The 3-hydroxy-4*H*-pyran-4-one moiety, contained in kojic acid, has been shown to serve as a catechol replacement for β-adrenergic agonist activity.[11] This group does not appear to have been investigated as a potential carboxyl equivalent. Its acidic pK_a of 7.9 indicated that the corresponding amine (kojic amine, **3**) would not exist in zwitterionic form. The synthesis and biological activity of this analogue, which is related to muscimol and GABA, are presented in this paper. A variety of derivatives and analogues of kojic amine is also described.

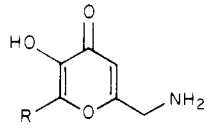
Chemistry. While the *N*,*N*-dimethyl derivative has been reported previously,[12] the primary amine 2-(aminomethyl)-5-hydroxy-4*H*-pyran-4-one (kojic amine, **3**) and its mono-*N*-methyl derivative were previously unknown. The synthesis of **3** was carried out by two different methods (Scheme I). In the preferred procedure, 2-(chloromethyl)-5-hydroxy-4*H*-pyran-4-one (or the corresponding tosylate ester) was converted to the azide **4**,[13] which on treatment with HBr in a mixture of acetic acid and phenol[14] gave an 81% yield of the primary amine (**3**). The latter could be isolated as a stable crystalline dihydrobromide salt (**3a**) from this reaction, but the less acidic monohydrobromide and hydrochloride salts were preferred for biological studies. Catalytic hydrogenolysis of the azide (**4**), using palladium, also gave **3**. In an alternative route, 5-(benzyloxy)-2-[(*p*-toluenesulfonyloxy)methyl]-4*H*-pyran-4-one (**5**),[15] when allowed to react with NH_3 at room temperature followed by debenzylation with HBr, gave a lower yield of **3**. A byproduct of this sequence was the disubstituted amine **6**.

Because of the encouraging biological activity of **3**, syntheses of a number of close analogues were carried out. The *N*-methyl and *N*-benzyl derivatives **7a** and **7b** were prepared as shown in Scheme I. The α-methyl derivative **8** was prepared starting from 5-(benzyloxy)-4-oxo-4*H*-pyran-2-carboxaldehyde[11] as shown in Scheme II. The isomeric 2-(aminomethyl)-3-hydroxy-4*H*-pyran-4-one (**9a**) was synthesized from 3-hydroxy-4*H*-pyran-4-one by a route paralleling that to **3** (Scheme III). An unexpected byproduct **10** resulted from the azide-forming reaction due to alkylation at the 2 position of the pyrone ring by a second mole of pyrone reagent. The 2 position in 3-hydroxy-4*H*-pyran-4-ones is known to be highly reactive to electrophilic substitution.

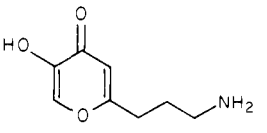
The structure of **10** was supported by a M^+ ion in the mass spectrum as well as by NMR evidence.[26] While reactions of this type do not appear to have been reported for this system, it is probably a general reaction. Three possibilities for the mechanism can be written:



A similar byproduct **10b** was obtained in the preparation of the 6-methyl derivative **9b**. The 6-bromo derivative 11 of kojic amine was prepared by halogenation of the azide **4** using NBS, followed by reduction. Nitration of the azide **4** occurred readily, but in attempting the HBr–phenol reduction the product was the 6-bromo derivative of kojic amine, 11. The ease of displacement of nitro by bromide was unexpected. Displacement of the nitro by other nucleophiles in this system may have general application but was not further studied. The 6-methyl derivative (**12**)



11, R = Br
12, R = CH_3



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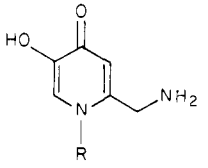
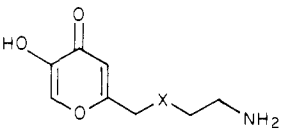
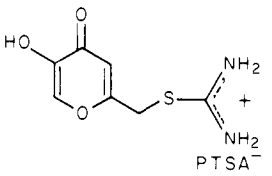


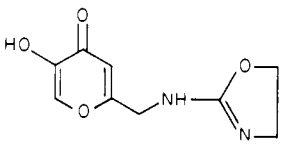
19a, R = H
b, R = CH_3



20a, X = S
b, X = SO_2



21



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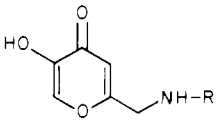


23, R = $COCH_2NH_2$
24, R = $COCH(NH_2)CH_3$
25, R = $COCH_2CH_2NH_2$
26, R = $COCH_2CH_2CH_2NH_2$
27, R = $COCH_2NHCOCH_2NH_2$
28, R = $COCH_3$

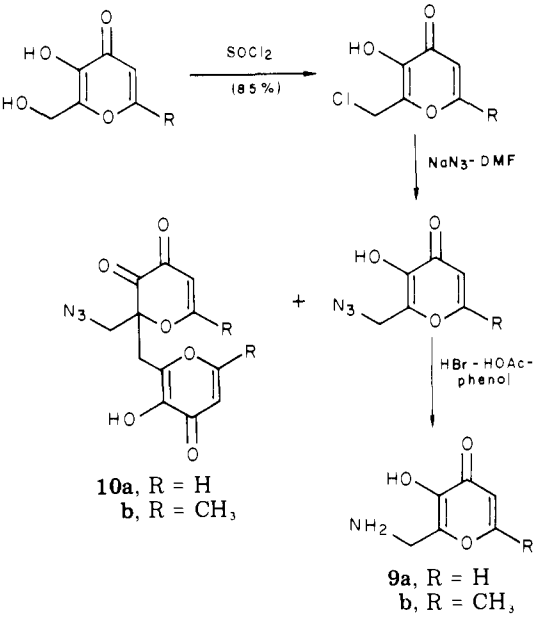
of kojic amine was synthesized from 6-methylkojic acid by the benzyl ether procedure described above for kojic amine.

The synthesis of the homologue **16** of kojic amine is outlined in Scheme IV. This compound was also of interest as a potential dopamine analogue. The major product of the reaction of **5** with cyanide was the trisubstituted acetonitrile derivative **14b**, along with small amounts of **14a** and **15**. The formation of **14b** underlines the ease of alkylation of the acetonitrile intermediate **15**. However, by using inverse addition under more dilute conditions, **15** was obtained in >90% yield. The latter was converted by hydrogenation with Raney nickel catalyst in acetic anhydride, followed by hydrolysis, to **16**. In an alternative approach, **5** when reacted with nitromethane gave, unexpectedly, the hydroxynitroethane derivative **17**.[11] This probably results from initial aldehyde formation[16] followed by condensation of the aldehyde and nitromethane. Compound **18**, with the chain extended to three carbons, was prepared by alkylation of ethyl cyanoacetate with **5**, followed by hydrolysis, decarboxylation, and reduction. Syntheses of the pyridine analogues of kojic amine, **19a** and **19b**, were carried out by a standard displacement of the ring oxygen with NH_3 and CH_3NH_2. Preparations of derivatives **20a,b**, **21**, and **22** were uncomplicated and are described in the Experimental Section.

The peptide-like derivatives of kojic amine, **23–27**, were prepared from 2-(aminomethyl)-5-(benzyloxy)-4*H*-pyran-4-one using either the *p*-nitrophenyl ester or the ethyl chloroformate procedure with the appropriate Boc-protected amino acid intermediate, followed by hydrolysis of the protecting groups.

Pharmacology. (A) Skeletal Muscle Relaxant Activity. Kojic amine effectively reduced flexor spasms in chronic spinal rat and cat preparations. In the rat, 70% reduction of flexor spasms occurred after a cumulative dose (ED_{70}) of 8 mg/kg iv of kojic amine. For muscimol, a similar reduction of flexor spasms was observed only after cumulative doses of 16 mg/kg iv, which were ultimately lethal. In the cat, ED_{70} for reduction of flexor spasms by kojic amine was 2.2 mg/kg iv, whereas doses up to 7.5 mg/kg iv of muscimol were ineffective. An ED_{70} of 4 mg/kg po obtained for kojic amine indicated good oral absorption in this preparation. Both the glycinamide derivative **23** and the alaninamide derivative **24** of kojic amine had ED_{70} values of about 8 mg/kg iv in the rat model and about 12 mg/kg iv in the cat.

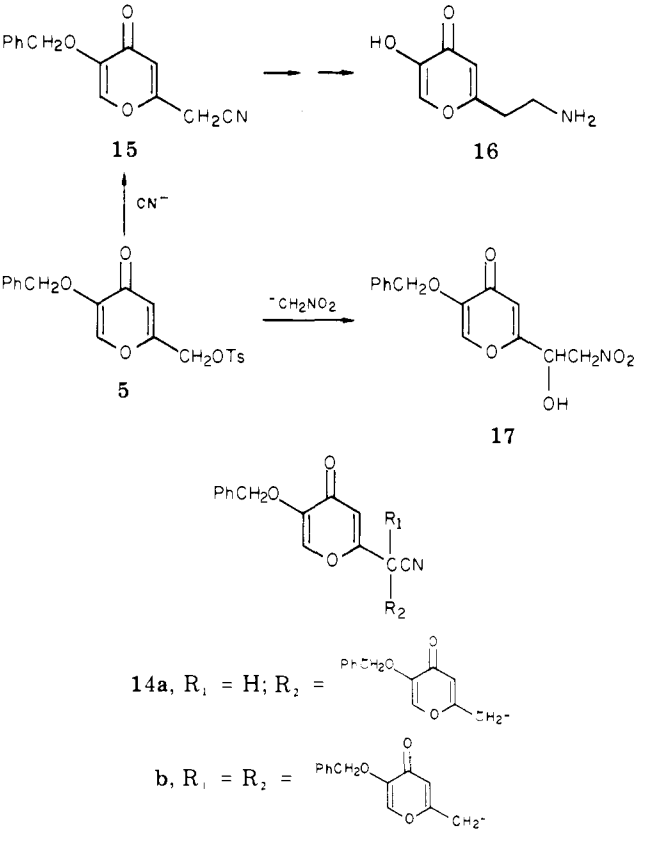
Scheme III



10a, R = H
b, R = CH_3

9a, R = H
b, R = CH_3

Scheme IV



15

16

5

17

14a, R_1 = H; R_2 =

b, R_1 = R_2 =

(B) Anticonvulsant Activity in Mice. Intraperitoneal injection of 3-mercaptopropionic acid has been shown to elicit a rapid and dramatic decrease in brain GABA levels immediately prior to the onset of tonic extensor seizures.[17] Oral doses required to prevent the induction of tonic extensor seizures in 50% of mice (ED_{50}) were 15 mg/kg for kojic amine and only 1.34 mg/kg for muscimol. However, in this species, kojic amine appears less toxic, since all animals survived acute oral doses of 128 mg/kg, whereas 8 mg/kg of muscimol was lethal to all mice.

Structure–Activity Relationships. 2-(Aminomethyl)-5-hydroxy-4*H*-pyran-4-one (kojic amine) was found to be orally active as a muscle relaxant in the cat flexor spasm procedure. Small changes in structure rendered the compound inactive. Thus, the *N*-methyl, *N*,*N*-dimethyl, *N*-benzyl, and *N*-acetyl (**28**) derivatives are all inactive. Introduction of a methyl group α to the amine or in the 6 position of the pyran ring, or of bromine or chlorine in the 6 position, all resulted in inactivity. The isomer in which the aminomethyl group is adjacent to the hydroxyl group is also devoid of activity, as are compounds in which the ring oxygen is replaced by NH or NCH_3. Homologue **16** was inactive in this assay and, in addition, showed no evidence of dopamine-like activity in the lesioned rat model.[18] Compound **18** with the chain extended to three carbons was also inactive up to the maximum dose of 16 mg/kg iv.

With this information available, several amino acid amide (or peptidelike) derivatives of kojic amine were examined. It was felt that the intact derivatives might show intrinsic CNS activity or that kojic amine, because of its ability to penetrate the CNS, might serve as a carrier of neurotransmitter amines, such as glycine and GABA, into the CNS. The *N*-glycyl derivative **23** and the *N*-α-alanyl derivative **24** did indeed show CNS activity, albeit less than that of kojic amine. It has not been determined whether the activity is due to hydrolysis to kojic amine. The lack of activity of the β-alanine and GABA derivatives **25** and **26** would indicate that hydrolysis of these derivatives in vivo does not occur to an appreciable extent.

The pK_a values of kojic amine were found to be 6.6 and 8.0, with the latter being attributed to the acidic hydroxyl group; a value of 7.9 has been reported for kojic acid,[19] and we obtained a value of 7.95. Thus, under physiological conditions, kojic amine would not be expected to have any zwitterionic character. This expectation is confirmed by examination of the ^{13}C NMR spectra of kojic amine and its hydrochloride salt (Table I). The effects of protonation of the amine on the chemical shift of the CH_2 group and the one-bond C–H coupling constant parallel those reported by Horsley and Sternlicht[20] in a study of a series of amino acids and indicate clearly that the nitrogen is not protonated in Me_2SO or aqueous Me_2SO solution. The essentially un-ionized character of kojic amine in solution may thus be a contributing factor to its ready penetration to the CNS.

The very narrow structure–activity picture in whole animal tests is perhaps not surprising in retrospect. Krogsgaard-Larsen and co-workers[21] have reported in vitro and microelectrophoretic studies of a series of muscimol analogues, and a relatively narrow structure–activity relationship is seen in these much simpler biological systems. Kojic amine has been shown to be a potent and specific inhibitor of sodium-independent [^{3}H]GABA binding to rat brain membranes,[22] and iontophoretic applications of kojic amine caused a pronounced, bicuculline sensitive, inhibition of the firing of spontaneously active cerebral cortical and cerebellar Purkinje cells in anesthetized rats.[23]

Table I. ^{13}C NMR Data for Kojic Acid[a] and Kojic Amine (3)

compd	chemical shifts[b]									
	C_2	C_3	C_4	C_5	C_6	C_7				
R = OH (kojic acid)	167.8	109.6	173.5	145.4	139.0	59.3				
R = NH_2 (3)	169.9	109.5	173.9	145.4	138.9	42.7				
						(43.1)[c]				
R = $^+NH_3$ (3·HCl)	159.9	113.5	173.4	146.0	139.8	39.1				
						(41.3)[c]				

compd	^{13}C–^1H coupling constants[b]									
	$^3J_{C_2-H_7}$	$^2J_{C_2-H_7}$	$^3J_{C_2-H_6}$	$^1J_{C_3-H_3}$	$^2J_{C_3-H_3}$	$^3J_{C_3-H_3}$	$^1J_{C_4-H_4}$	$^2J_{C_5-H_4}$	$^3J_{C_5-H_5}$	$^1J_{C_6-H_6}$	$^1J_{C_7-H_7}$	$^2J_{C_7-H_7}$
R = OH (kojic acid)	4.7	4.7	7.3	166.5	2.6	1.5	5.1	3.5	5.4	197.5	143.0[d]	2.0[d]
R = NH_2 (3)	5.6	5.6	6.0	166.2	2.5	1.5	5.4	3.5	5.5	197.8	135.6	2.0
											(138.5)[c]	
R = $^+NH_3$ (3·HCl)	5.4	5.4	7.4	168.0	2.3	0	6.0	3.5	5.1	199.2	145.1	2.0
											(146.1)[c]	

[a] Vs. Me_2SO-d_6, taken as 39.4 ppm from Me_4Si. [b] Approximate value due to peak broadening. [c] Values observed in H_2O–Me_2SO-d_6 (2:1). [d] This work.

Thus, kojic amine constitutes a novel structure possessing many of the properties, both in vivo and in vitro, to be expected of a GABA-like compound. Study of its properties and applications are continuing.

Experimental Section

Melting points were determined using a Thomas-Hoover melting-point apparatus and are uncorrected. ^{1}H NMR 60-MHz spectra were recorded with a Varian Associates EM-360 instrument. Chemical shifts were recorded in parts per million (δ) relative to Me_4Si as an internal standard. ^{13}C NMR spectra were obtained on a Varian CFT-20 spectrometer, and the chemical shifts are reported relative to internal Me_4Si. IR spectra were determined on a Perkin-Elmer 257 spectrophotometer. Mass spectral analyses were provided by Morgan-Schaffer Corp., Montreal, and elemental analyses by Dr. C. Daessle, Montreal. The ^{13}C NMR and pK_a determinations were performed by Mr. S. C. Ho and associates at Merck Frosst Laboratories.

2-(Aminomethyl)-5-hydroxy-4*H*-pyran-4-one (Kojic Amine, 3). Method A. A mixture of 2-(azidomethyl)-5-hydroxy-4*H*-pyran-4-one (**4**)[13] (143 g, 0.86 mol), mp 128–130 °C, and phenol (72 g) was dissolved in 700 mL of HOAc. The solution was cooled in an ice bath and saturated with HBr gas. The mixture, after being stirred for 3.5 h, was cooled with ice and filtered. The solid was washed with a small quantity of HOAc and then freely with THF and then Et_2O. Crude **3a** (287 g) was obtained: mp 206–207 °C; ^{1}H NMR (Me_2SO-d_6) δ 4.2 (2 H, quartet, CH_2, collapses to a singlet on D_2O exchange, $J_{CH_2\text{-}NH_3^+}$ = 5 Hz), 6.68 (1 H, s, H_3), 8.22 (1 H, s, H_6), 8.72 (3 H, br, $^+NH_3$), 9.45 (2 H, s, 2OH). Anal. Calcd for $C_6H_9Br_2NO_3$: C, 23.78; H, 2.99; N, 4.62; Br, 52.76. Found: C, 24.80; H, 3.44; N, 5.58; Br, 49.54.

3a (287 g) was dissolved in MeOH (1500 mL), and THF (1000 mL) was added. The solution was concentrated to 500 mL, and 1000 mL of THF was added. On filtration, **3**·HBr (154 g, 81%) was obtained: mp 220–222 °C; ^{1}H NMR (Me_2SO-d_6) δ 4.10 (2 H, s, CH_2), 6.68 (1 H, s, H_3), 8.22 (1 H, s, H_6), 8.65 (4 H, br s, OH and NH_3^+). Anal. ($C_6H_8BrNO_3$) C, H, Br, N.

Metathesis using Amberlite AG 1-X8 resin (Cl^- cycle) gave **3**·HCl: mp 240–245 °C; ^{1}H NMR (Me_2SO-d_6) δ 4.02 (2 H, s, CH_2), 6.65 (1 H, s, H_3), 8.12 (1 H, s, H_6), 9.10 (4 H, br s, OH and $^+NH_3$). Anal. ($C_6H_8ClNO_3$) C, H, Cl, N.

An aqueous solution of kojic amine hydrobromide was passed through a column of AG 50W-X8 resin (acid cycle) and eluted with 1 N NH_4OH. Upon concentration of the eluate, free kojic amine (**3**) crystallized from the aqueous solution: mp 181 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 3.57 (2 H, s, CH_2), 5.58 (3 H, br s, OH and NH_2), 6.43 (1 H, s, H_3), 8.02 (1 H, s, H_6); IR (KBr) 3200–2150 (br, OH–NH_2), 1630, 1595, 1560 cm^{-1} (strong, C=O, C=C). Anal. ($C_6H_7NO_3$) C, H, N.

The ^{13}C NMR spectral data of **3** and **3**·HCl are collected in Table I, along with the published data[24] for kojic acid.

Method B. Into a refluxing solution of **5** (500 g, 1.26 mol) in $CHCl_3$ (5.5 L)–MeOH (3.5 L) was passed NH_3 gas for 9 h. The solvent was evaporated and the residue slurried with $CHCl_3$ (1.5 L) and filtered to give 240 g of a mixture of salts. These solids were dissolved in H_2O (1.0 L), and the solution was made basic with K_2CO_3 and then extracted with $CHCl_3$. The $CHCl_3$ extract on evaporation gave 2-(aminomethyl)-5-(benzyloxy)-4*H*-pyran-4-one (27.6 g). The $CHCl_3$ solution from the initial slurry was shaken with 1 N HCl (1.5 L) and the salt which formed filtered (80 g). The solid was partitioned between $CHCl_3$ and aqueous K_2CO_3 solution. The $CHCl_3$ solution on evaporation afforded bis[5-(benzyloxy)-4-oxo-4*H*-pyran-2-yl]methylamine, mp 163–165 °C (60 g). Anal. ($C_{20}H_{23}NO_6$) C, H, N.

The aqueous HCl phase, which was made basic with aqueous K_2CO_3 and extracted with $CHCl_3$, provided an additional 79 g of 2-(aminomethyl)-5-(benzyloxy)-4*H*-pyran-4-one (total yield 106.6 g, 38%), mp 84.5–86.5 °C. Anal. ($C_{13}H_{13}NO_3$) C, H, N.

A solution of 2-(aminomethyl)-5-(benzyloxy)-4*H*-pyran-4-one (8.9 g, 0.038 mol) in HOAc (200 mL) was made strongly acidic with anhydrous HBr and then heated at 100 °C for 20 min. On cooling there was obtained **3a**. Recrystallization from MeOH–THF gave **3**·HBr.

A similar HBr–HOAc treatment of the crude bis[5-(benzyloxy)-4-oxo-4*H*-pyran-2-yl]methylamine gave **6**·HBr: mp 270–275 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 4.36 (4 H, s, $2CH_2$), 6.77 (2 H, s, $2H_3$), 6.5–8.0 (4 H, br s, 2OH and $^+NH_2$), 8.27 (2 H, s, $2H_6$). Anal. ($C_{12}H_{12}BrNO_6$) C, H, Br, N.

5-Hydroxy-2-[(methylamino)methyl]-4*H*-pyran-4-one (7a). The procedure of method B, when repeated using methylamine in place of NH_3, gave **7a**·HBr·H_2O: mp 187–188 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.65 (3 H, s, CH_3), 4.26 (2 H, s, CH_2), 6.73 (1 H, s, H_3), 8.27 (1 H, s, H_6), 9.42 (3 H, br s, OH and $^+NH_2$). Anal. ($C_7H_{12}BrNO_4$) C, H, Br, N.

2-[(Benzylamino)methyl]-5-hydroxy-4*H*-pyran-4-one (7b). When benzylamine was substituted for methylamine in the above procedure, **7b**·HBr was obtained: mp 270–273 °C; ^{1}H NMR (Me_2SO-d_6) δ 4.22 and 4.66 (4 H, 2 s, $CH_2NH_2^+CH_2Ph$), 6.66 (1 H, s, H_3), 7.25–7.70 (5 H, m, C_6H_5), 8.21 (1 H, s, H_6), 8.0–10.0 (3 H, br s, OH and NH_2^+). Anal. ($C_{13}H_{14}BrNO_3$) C, H, Br, N.

DL-2-(1-Aminoethyl)-5-hydroxy-4*H*-pyran-4-one (8). To a suspension of 5-(benzyloxy)-4-oxo-4*H*-pyran-2-carboxaldehyde[11] (35 g, 0.152 mol) in THF (1.0 L) was added in portions a 1.5 M solution of MeMgI (80 mL, 0.12 mol). After the mixture was agitated for 0.5 h, H_2O (200 mL) was added to decompose the complex. Following evaporation, the residue was extracted with CH_2Cl_2 (3 × 200 mL). The extract was concentrated and the residue reextracted with EtOAc and with $CHCl_3$. The oil (20 g) obtained on evaporation of the combined extracts was chromatographed (silica gel, EtOAc–C_6H_6, 1:1) to give as an oil (5.5 g, 14.7%) 2-(1-hydroxyethyl)-5-(benzyloxy)-4*H*-pyran-4-one. A mixture of this intermediate (5.0 g, 0.02 mol) and $Na_2Cr_2O_7$ (5.0 g, 0.02 mol) in HOAc (50 mL) was heated for 10 min at 100 °C. Ice was added and the mixture extracted with $CHCl_3$. The $CHCl_3$ extract, after washing with H_2O and drying over $MgSO_4$, was evaporated to yield 4.0 g (80%) of 2-acetyl-5-(benzyloxy)-4*H*-pyran-4-one, mp 145–147 °C. Anal. ($C_{14}H_{12}O_4$) C, H.

A mixture of this intermediate (3.8 g, 0.015 mol), NH_2OH·HCl (6.0 g, 0.086 mol), and *N*-ethylpiperidine (9.0 g) in MeOH (60 mL) was stirred for 2 h. Addition of ice, followed by filtration, gave 2.3 g (59%) of solid oxime, mp 220–221 °C, which was not further purified. A mixture of oxime (2.1 g, 0.008 mol), Zn (2.1 g), and HOAc was stirred for 6 h. After filtration of inorganic solids and evaporation of the filtrate, the residue was slurried with Et_2O to give 2.46 g of solid amine acetate. To the solid was added 48% HBr (80 mL), and the mixture was heated at 110–120 °C for 0.25 h. The cooled solution was extracted with Et_2O. The aqueous portion was evaporated to an oil which was dissolved in H_2O and passed through a resin column (AG 50W-X8, acid cycle). The residue from evaporation of the eluate was recrystallized from EtOH to give **8** (0.52 g, 42%): mp 170–174 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.24 (3 H, d, CH_3, J_{CH_3CH} = 7 Hz), 3.78 (1 H, quartet, CH, J_{CHCH_3} = 7 Hz), 4.12 (3 H, br s, OH and NH_2), 6.47 (1 H, s, H_3), 8.07 (1 H, s, H_6). Anal. ($C_7H_9NO_3$) C, H, N.

2-(Aminomethyl)-3-hydroxy-4*H*-pyran-4-one (9a). A mixture of 2-(chloromethyl)-3-hydroxy-4*H*-pyran-4-one[25] (2.6 g, 0.016 mol) and NaN_3 (1.1 g, 0.016 mol) in DMF (30 mL) was stirred for 18 h at 20 °C. Following filtration and evaporation of the filtrate, the residue was extracted with $CHCl_3$. On evaporation there was obtained a roughly 1:1 mixture of **10a** and 2-(azidomethyl)-3-hydroxy-4*H*-pyran-4-one, as shown by NMR, TLC, and mass spectral evidence.

This mixture (0.88 g) in HOAc (20 mL) was saturated with gaseous HBr with ice cooling. After 2 h, **9a**·HBr was filtered (0.52 g, 14.6%): mp 208–210 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 4.06 (2 H, br s, CH_2), 6.45 (1 H, d, H_5, $J_{H_5\text{-}H_6}$ = 6 Hz), 8.20 (1 H, d, H_6, $J_{H_6\text{-}H_5}$ = 6 Hz), 8.50 (4 H, br s, OH and $^+NH_3$). Anal. ($C_6H_8BrNO_3$) C, H, Br, N.

2-(Aminomethyl)-3-hydroxy-6-methyl-4*H*-pyran-4-one (9b). When the previous procedure was repeated starting from 2-(hydroxymethyl)-3-hydroxy-6-methyl-4*H*-pyran-4-one, **9b**·HBr was obtained in 27% overall yield: mp 270–275 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 2.32 (3 H, s, CH_3), 4.10 (2 H, br s, CH_2), 6.40 (1 H, s, H_5), 8.5 (4 H, br s, OH and $^+NH_3$). Anal. ($C_7H_{10}BrNO_3$) C, H, Br, N.

In the azide-forming step, the byproduct **10b**[26] was obtained: mp 179–181 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.03 and 2.30 (6 H, 2 s, $2CH_3$), 3.17 (2 H, br s, $-CH_2-$), 3.43 and 4.15 (2 H, dd, $-CH_2N_3$, J_{gem} = 14 Hz), 5.70 and 6.30 (2 H, 2 s, $2H_5$), 8.53 (1 H, s, OH); IR (KBr) 2100 (strong, N_3), 1695, 1670, 1650–1620 cm^{-1} (strong, C=O and C=C); MS (M^+ 319). Anal. Calcd for $C_{14}H_{13}N_3O_6$:

C, 52.66; H, 4.10; N, 13.16. Found: C, 52.51; H, 4.57; N, 12.89.

6-(Aminomethyl)-2-bromo-3-hydroxy-4*H*-pyran-4-one (11). A mixture containing 4 (10 g, 0.06 mol) and *N*-bromosuccinimide (12 g, 0.067 mol) in benzene (150 mL) was refluxed for 1 h. After filtration (while hot) and evaporation, 13 g (88%) of 6-(azidomethyl)-2-bromo-3-hydroxy-4*H*-pyran-4-one was obtained, mp 112–114 °C. Anal. ($C_6H_4BrN_3O_3$) C, H, Br, N.

A mixture of this azide (5 g, 0.02 mol) and phenol (2.5 g) in HOAc (35 mL) was saturated with HBr with ice cooling. After standing overnight, the solids were filtered (5.6 g) and dissolved in MeOH, and the MeOH solution was evaporated to a small volume to give 3.7 g of **11**·HBr (61.5%): mp 300 °C; ^{1}H NMR (Me_2SO-d_6) δ 4.15 (2 H, s, CH_2), 6.73 (1 H, s, H_5), 8.95 (4 H, br s, OH and $^+NH_3$). Anal. ($C_6H_7Br_2NO_3$) C, H, Br, N.

6-(Aminomethyl)-3-hydroxy-2-methyl-4*H*-pyran-4-one (12). **12**·HBr, mp 241–242 °C, was prepared in 48% overall yield by method B starting from 6-(hydroxymethyl)-3-hydroxy-2-methyl-4*H*-pyran-4-one:[25] ^{1}H NMR (Me_2SO-d_6) δ 2.30 (3 H, s, CH_3), 4.10 (2 H, s, CH_2), 6.63 (1 H, s, H_5), 8.77 (4 H, br s, OH and $^+NH_3$). Anal. ($C_7H_{10}BrNO_3$) C, H, Br, N.

2-(2-Aminoethyl)-5-hydroxy-4*H*-pyran-4-one (16). A mixture of **5** (78 g, 0.2 mol) and NaCN (9.8 g, 0.2 mol) in DMF (300 mL) was allowed to react at 0 °C for 1 h and then quenched with ice. The red gum which formed after washing with H_2O was triturated with MeOH. On filtration the trisubstituted acetonitrile derivative **14b** (15 g) was obtained and was recrystallized from *i*-PrOH: mp 204–205 °C; ^{1}H NMR (Me_2SO-d_6) δ 3.60 (4 H, br s, 2-CH_2-), 5.00 (4 H, s, 2-CH_2Ph), 5.08 (2 H, s, -CH_2Ph), 6.47 (2 H, s, $2H_3$), 6.65 (1 H, s, H_3), 7.50 (15 H, s, $3C_6H_5$), 8.27 (2 H, s, $2H_6$), 8.42 (1 H, s, H_6); IR (KBr) 1650 cm^{-1} (C=O); MS (M^+ 669). Anal. Calcd for $C_{40}H_{31}NO_9$: C, 71.74; H, 4.67; N, 2.09. Found: C, 71.52; H, 4.81; N, 2.02.

Fractions, obtained by evaporation of the MeOH filtrate and $CHCl_3$ extraction of the initial aqueous phase and washings, were combined and chromatographed [silica gel, EtOAc–C_6H_6 (1:10, 1:4), and then *i*-PrOH–EtOAc (1:10)] to give 6 g of recovered starting tosylate, 2.83 g of [5-(benzyloxy)-4-oxo-4*H*-pyran-2-yl]acetonitrile (**15**) [mp 150–151 °C; ^{1}H NMR ($CDCl_3$) δ 3.68 (2 H, s, CH_2CN), 5.15 (2 H, s, CH_2Ph), 6.57 (1 H, s, H_3), 7.50 (5 H, s, C_6H_5), 7.67 (1 H, s, H_6); MS (M^+ 241); IR (KBr) 2260 cm^{-1} (weak, CN), 1650–1620 cm^{-1} (s, C=O and C=C). Anal. Calcd for $C_{14}H_{11}NO_3$: C, 69.70; H, 4.59; N, 5.80. Found: C, 69.78; H, 4.44; N, 5.79], and, finally, 3.13 g of disubstituted acetonitrile derivative **14a** [mp 164–166 °C; ^{1}H NMR (Me_2SO-d_6) δ 3.40 (2 H, m, -CH_2-), 5.00 (5 H, br s, 2-CH_2Ph and -CH-), 6.53 and 6.62 (2 H, 2 s, $2H_3$), 7.50 (10 H, s, $2C_6H_5$), 8.30 and 8.42 (2 H, 2 s, $2H_6$); IR (KBr) 1650 cm^{-1} (C=O); MS (M^+ 455). Anal. Calcd for $C_{27}H_{21}NO_6$: C, 71.20; H, 4.65; N, 3.08. Found: C, 70.99; H, 4.60; N, 2.92.].

The intermediate **15** (1.9 g, 0.008 mol) in Ac_2O (250 mL) containing 1.0 g of Raney nickel was hydrogenated in a Parr low-pressure apparatus for 2 h. Filtration and evaporation gave an oil (2.1 g) which by NMR was a mixture of 2-(2-acetamidoethyl)-5-acetoxy-4*H*-pyran-4-one and 2-(2-acetamidoethyl)-5-(benzyloxy)-4*H*-pyran-4-one. The oil (2.1 g) in 20% HCl (150 mL) was refluxed for 2 h. After evaporation, addition of *i*-PrOH and reevaporation were repeated several times to give **16**·HCl (0.83 g, 55%): mp 207–209 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 3.05 (4 H, br s, CH_2CH_2), 6.40 (1 H, s, H_3), 8.13 (1 H, s, H_6), 8.45 (4 H, br s, OH and $^+NH_3$). Anal. ($C_7H_{10}ClNO_3$) C, H, Cl, N.

By addition of tosylate **5** to a 30-fold excess of a 1.35 M NaCN solution in HMPT, the desired acetonitrile derivative **15** was obtained in >90% yield, containing only traces of the dialkylated compound **14a**.

2-(1-Hydroxy-2-nitroethyl)-5-(benzyloxy)-4*H*-pyran-4-one (17). To 15 mL of nitromethane stirred at 20 °C was added in portions 3 g (0.125 mol) of NaH (**caution**: the sodium salt of nitromethane can decompose violently). After reaction was complete, the white paste was diluted with 200 mL of HMPT and cooled in an ice bath, and 40 g (0.1 mol) of tosylate **5** was added. After 2 h of stirring, the reaction was complete (TLC) and was diluted with 1200 mL of H_2O. Extraction with 1:1 Et_2O–EtOAc, drying, and evaporation left 20 g of a semisolid residue which by TLC contained traces of **5**, a major, more polar component, and a minor, still more polar material. Slurrying of this residue with 50 mL of $CHCl_3$ gave rise to insoluble fine crystals, which were filtered and washed with Et_2O to give 5.1 g (0.017 mol) of **17**, identical with an authentic sample.[11]

3-(5-Hydroxy-4-oxo-4*H*-pyran-2-yl)propylamine (18). Tosylate **5** (40 g, 0.104 mol) was added to a mixture of the potassium salt of ethyl cyanoacetate [prepared from ethyl cyanoacetate (80 g, 0.71 mol) and potassium *tert*-butoxide (80 g, 0.71 mol)] in DMF (2.8 L). After being stirred for 2–3 min, the mixture was poured into ice–H_2O. Extraction with $CHCl_3$ afforded an oil which was chromatographed (silica gel, C_6H_6 and then EtOAc) to give ethyl 2-cyano-3-[5-(benzyloxy)-4-oxo-4*H*-pyran-2-yl]-propionate, mp 65–67 °C (18 g, 53%). Anal. ($C_{18}H_{17}NO_5$) C, H, N.

Dialkylation occurred to a significant extent if a smaller ratio of ethyl cyanoacetate was used in this reaction.

A mixture of ethyl 2-cyano-3-[5-(benzyloxy)-4-oxo-4*H*-pyran-2-yl]propionate (12.0 g, 0.037 mol) and KOH (2.06 g, 0.036 mol) in MeOH (50 mL) was stirred for 7 days. Evaporation to dryness, followed by trituration with Et_2O, gave the potassium salt which was dissolved in H_2O and converted to the insoluble free acid (8.3 g, 75%), mp 132–135 °C, by acidification with dilute HCl. Anal. ($C_{16}H_{13}NO_5$·0.5H_2O) C, H, N.

Heating 8.3 g (0.028 mol) of the above acid at 140–145 °C under vacuum until CO_2 evolution ceased (2 h), followed by dissolution in $CHCl_3$, and decolorization with charcoal, gave a solid residue. Trituration with Et_2O left 4.8 g (67.2%) of 3-[5-(benzyloxy)-4-oxo-4*H*-pyran-2-yl]propionitrile. A mixture of the nitrile (1.76 g, 0.067 mol) in Ac_2O (150 mL) with Raney nickel catalyst (0.5 g) was hydrogenated in a Parr low-pressure hydrogenator for 1.5 h. After filtration and evaporation to dryness, the residue was dissolved in toluene and reevaporated to give 1.5 g (71%) of crude acetylated amine.

The crude amide in concentrated HCl (60 mL) was heated at 130 °C for 5 h to effect hydrolysis. After evaporation, the residue was neutralized with dilute NH_4OH, and the solution was passed through a column of Amberlite 50W-X8 resin. The eluate was evaporated and the residue slurried with H_2O and filtered to give 0.25 g (19%) of **18**·H_2O: mp 143–145 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.70 (2 H, quintet, -$CH_2CH_2CH_2NH_2$), 2.58 (4 H, m, -$CH_2CH_2CH_2NH_2$), 4.87 (3 H, s, OH and NH_2), 6.23 (1 H, s, H_3), 7.97 (1 H, s, H_6). Anal. ($C_8H_{13}NO_4$) C, H, N.

2-(Aminomethyl)-5-hydroxy-4(1*H*)-pyridinone (19a). 2-(Aminomethyl)-5-(benzyloxy)-4*H*-pyran-4-one (5 g, 0.022 mol) in Ac_2O (100 mL) was heated on a steam bath for 20 min. The residue from evaporation was slurried with Et_2O and filtered to give 5.8 g (96.5%), mp 137–139 °C, of the *N*-acetyl derivative. Anal. ($C_{15}H_{15}NO_4$) C, H, N.

A mixture of this intermediate (5 g, 0.018 mol), concentrated NH_4OH (200 mL), and dioxane (75 mL) was heated to reflux for 8 h with continuous passage of ammonia gas. Following evaporation, the residue was slurried with hot EtOAc (50 mL) to remove traces of starting material and filtered to yield 4.67 g (93%) of 2-(acetamidomethyl)-5-(benzyloxy)-4(1*H*)-pyridinone, mp 217–220 °C. Anal. ($C_{15}H_{16}N_2O_3$) C, H, N.

A mixture of this intermediate (1.5 g, 5.5 mol) in 48% HBr (120 mL) was refluxed for 3 h. The reaction mixture was evaporated and the residue taken up in 100 mL of H_2O, treated with charcoal, filtered, and evaporated. The oily residue was slurried with 50 mL of THF, which gave a crystalline solid. Filtration yielded 1.11 g (65%) of **19a** as the dihydrobromide hydrate: mp 220–225 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 4.30 (2 H, s, CH_2), 7.50 (1 H, s, H_3), 8.26 (1 H, s, H_6), 9.60 (6 H, br s, 2OH, ^+NH and $^+NH_3$). Anal. ($C_6H_{12}Br_2N_2O_3$) C, H, Br, N.

1-Methyl-2-(aminomethyl)-5-hydroxy-4(1*H*)-pyridinone (19b). When the preceding procedure was followed using aqueous methylamine instead of ammonia, there was obtained a mixture of mono- and dihydrobromide salts. Heating at 180 °C under vacuum for 18 h converted the mixture cleanly to the monohydrobromide of **19b**, mp 264–267 °C dec, which still retained H_2O of hydration: ^{1}H NMR (Me_2SO-d_6) δ 3.75 (3 H, s, CH_3), 4.18 (2 H, s, CH_2), 5.65 (4 H, br s, OH and $^+NH_3$), 6.46 (1 H, s, H_3), 7.67 (1 H, s, H_6). Anal. ($C_7H_{15}BrN_2O_4$) C, H, Br, N.

2-[(5-Hydroxy-4-oxo-4*H*-pyran-2-yl)methylthio]ethylamine (20a). To 50 mL of 2 N NaOH was added 11.3 g (0.1 mol) of 2-mercaptoethylamine hydrochloride at 0 °C. To this solution was added a solution of 18 g (0.106 mol) of benzyl chloroformate in 50 mL of toluene with vigorous stirring, followed by 25 mL of 4 N NaOH. After 15 min of stirring, 20 mL of Et_2O was added,

and the organic layer was separated and extracted with 1 N NaOH. The aqueous extract was acidified and extracted with Et_2O, and upon evaporation and trituration with petroleum ether there was obtained 15 g (0.07 mol, 70%) of *N*-(benzyloxycarbonyl)-mercaptoethylamine, mp 43 °C. Anal. ($C_{10}H_{13}NO_2S$) C, H, N, S.

To *N*-(benzyloxycarbonyl)mercaptoethylamine (12.66 g, 0.06 mol) in DMF (170 mL) was added potassium *tert*-butoxide (6.78 g, 0.06 mol). After complete solution occurred (15 min), to the mixture, cooled in ice, was added **5** (23.16 g, 0.06 mol). After stirring for 1.5 h, the reaction was quenched with ice and H_2O. The solid which formed, after washing with H_2O and drying, weighed 22 g (86.3%). The crude *N*-(benzyloxycarbonyl)-2-[[5-(benzyloxy)-4-oxo-4*H*-pyran-2-yl]methylthio]ethylamine (8 g, 0.019 mol) in concentrated HCl (200 mL) was heated at reflux for 1 h to effect hydrolysis. The aqueous phase, after extraction with Et_2O, was evaporated to dryness, slurried with EtOH overnight, and filtered to give 4.0 g (88.6%) of **20a**·HCl: mp 202–205 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.90 (4 H, m, CH_2CH_2), 3.76 (2 H, s, CH_2), 6.50 (1 H, s, H_3), 8.10 (1 H, s, H_6), 8.33 (4 H, br s, OH and $^+NH_3$). Anal. ($C_8H_{12}ClNO_3S$) C, H, Cl, N, S.

The sulfone **20b** was prepared by oxidation of the protected intermediate with H_2O_2–AcOH followed by hydrolysis as described above to yield the HCl salt: mp 222–224 °C; ^{1}H NMR (Me_2SO-d_6) δ 3.10–3.95 (4 H, m, CH_2CH_2), 4.93 (2 H, s, CH_2), 6.68 (1 H, s, H_3), 8.29 (1 H, s, H_6), 8.60 (4 H, br s, OH and $^+NH_3$). Anal. ($C_8H_{12}ClNO_5S$) C, H, Cl, N, S.

S-[(5-Hydroxy-4-oxo-4*H*-pyran-2-yl)methyl]thiouronium Tosylate (21). A mixture of **5** (38.6 g, 0.1 mol) and thiourea (8.2 g, 0.11 mol) in EtOH (250 mL) was refluxed for 0.5 h. On cooling, the product was filtered (40 g, 87%), mp 175–176 °C. The crude product was dissolved in concentrated HCl (400 mL), and the mixture was heated at 90–100 °C for 5 min. After cooling and extraction with Et_2O, the aqueous phase was evaporated. On flushing several times with H_2O and finally slurrying with about 150 mL of H_2O, 21.6 g of **21**·tosylate, mp 190–192 °C, was obtained: ^{1}H NMR (Me_2SO-d_6) δ 4.53 (2 H, s, CH_2), 6.56 (1 H, s, H_3), 8.20 (1 H, s, H_6), 9.40 (5 H, br s, OH and $-C(=NH_2^+)NH_2$); tosylate δ 2.33 (3 H, s, CH_3), 7.22 (2 H, d, H_3 and H_5), 7.62 (2 H, d, H_2 and H_6, $J_{2,3} = J_{5,6} = 8$ Hz). Anal. ($C_{14}H_{16}N_2O_6S_2$) C, H, N, S.

2-[[(5-Hydroxy-4-oxo-4*H*-pyran-2-yl)methyl]amino]-Δ^2-oxazoline (22). To a solution of 2-(aminomethyl)-5-(benzyloxy)-4*H*-pyran-4-one (9.24 g, 0.04 mol) in $CHCl_3$ (70 mL) was added chloroethyl isocyanate (4.8 g, 0.046 mol) in Et_2O (20 mL). After 15 min, Et_2O (800 mL) was added and filtration yielded 12.4 g (89.4%) of 1-[[5-(benzyloxy)-4-oxo-4*H*-pyran-2-yl]-methyl]-3-(2-chloroethyl)urea, mp 150–155 °C. Anal. ($C_{16}H_{17}ClN_2O_4$) C, H, Cl, N.

A mixture of the above urea (12.0 g, 0.035 mol), KI (12 g), H_2O (250 mL), and acetone (100 mL) was refluxed for 18 h. After evaporation, the residue was dissolved in H_2O and extracted with $CHCl_3$. The aqueous layer, after being made basic with NH_4OH, was extracted with $CHCl_3$. The $CHCl_3$ extract on evaporation afforded a solid which, after slurrying with Et_2O and filtration, yielded 2.1 g (20%) of 2-[[[5-(benzyloxy)-4-oxo-4*H*-pyran-2-yl]methyl]amino]-Δ^2-oxazoline, mp 123–125 °C. Anal. ($C_{16}H_{16}N_2O_4$) C, H, N.

This intermediate (2.1 g, 0.007 mol) in EtOH (25 mL) with 0.4 g of Pd/*C* catalyst was hydrogenated until 1 mol of H_2 was absorbed (2 h) in a Parr low-pressure hydrogenator. Filtration, followed by evaporation to dryness and slurrying with $CHCl_3$, afforded 0.39 g (26.5%) of **22**: mp 210–230 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 3.58 and 4.24 (4 H, m, $-CH_2CH_2-$), 4.10 (2 H, s, CH_2), 4.90 (2 H, br s, OH and NH), 6.27 (1 H, s, H_3), 8.06 (1 H, s, H_6). Anal. ($C_9H_{10}N_2O_4$) C, H, N.

2-Amino-*N*-[(5-hydroxy-4-oxo-4*H*-pyran-2-yl)methyl]-acetamide (23). 2-(Aminomethyl)-5-(benzyloxy)-4*H*-pyran-4-one (5 g, 0.022 mol) and *N*-(benzyloxycarbonyl)glycine *p*-nitrophenyl ester (7.5 g, 0.023 mol) in *i*-PrOH (120 mL) were refluxed for 20 min. The solid which formed was filtered while hot, yielding 7 g (75.4%), mp 179–180 °C, of 2-(benzyloxycarbonyl)amino-*N*-[[5-(benzyloxy)-4-oxo-4*H*-pyran-2-yl]methyl]acetamide intermediate. Anal. ($C_{23}H_{22}N_2O_6$) C, H, N.

A solution of this intermediate (5 g) in 70 mL of HOAc was saturated with HBr and refluxed for 30 min. **23**·2HBr was isolated by filtration (4 g). Two recrystallizations from MeOH afforded 2.23 g of **23**·HBr: mp 216–218 °C; ^{1}H NMR (Me_2SO-d_6) δ 3.77 (2 H, br s, $CH_2NH_3^+$), 4.30 (2 H, d, CH_2, collapses to a singlet on D_2O exchange, $J_{CH_2NH} = 6$ Hz), 6.44 (1 H, s, H_3), 8.17 (5 H, br s, H_6, OH and $^+NH_3$), 9.13 (1 H, triplet, NH, $J_{NHCH_2} = 6$ Hz). Anal. ($C_8H_{11}BrN_2O_4$) C, H, Br, N.

2-Amino-*N*-[(5-hydroxy-4-oxo-4*H*-pyran-2-yl)methyl]-propionamide (24). To a mixture of *N*-(benzyloxycarbonyl)-α-alanine (6.55 g, 0.029 mol), methylene chloride (60 mL), and Et_3N (4.5 mL) cooled to −5 °C was added ethyl chloroformate (2.8 mL), and the mixture was stirred for 5 min. To this solution was added a mixture of 2-(aminomethyl)-5-(benzyloxy)-4*H*-pyran-4-one (6.56 g, 0.029 mol), triethylamine (12 mL), and CH_2Cl_2 (60 mL). After being stirred for 15 min at −5 °C and 3 h at 20 °C, the reaction mixture was extracted with dilute HCl, $NaHCO_3$ solution, and H_2O. The organic layer was evaporated to dryness, and the residue was slurried with Et_2O and filtered to yield 7.75 g (64%) of 2-(benzyloxycarbonyl)amino-*N*-[[5-(benzyloxy)-4-oxo-4*H*-pyran-2-yl]methyl]propionamide, mp 127–128 °C. Anal. ($C_{24}H_{24}N_2O_6$) C, H, N.

The intermediate (6 g, 0.014 mol) dissolved in HOAc (120 mL) was saturated with HBr and the mixture heated at 90 °C for 25 min. After evaporation to dryness and flushing with HOAc, the residue was triturated with Et_2O and then H_2O to yield 0.9 g of starting amine which was discarded. The filtrate was neutralized with NaOH and passed through an Amberlite 50W-X8 resin. The eluate was evaporated and the residue slurried with EtOH to yield a solid which after filtration gave 2.22 g of **24** (74.8%): mp 175–178 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 1.16 (3 H, d, CH_3, $J_{CH_3CH} = 7$ Hz), 3.40 (1 H, quartet, CH, $J_{CHCH_3} = 7$ Hz), 4.20 (6 H, br s, CH_2, NH, OH, and NH_2), 6.30 (1 H, s, H_3), 8.06 (1 H, s, H_6). Anal. ($C_9H_{12}N_2O_4$) C, H, N.

When the above reaction sequence was repeated using the *N*-(benzyloxycarbonyl) derivatives of β-alanine and γ-aminobutyric acid, compounds **25** [mp >200 °C dec; ^{1}H NMR (D_2O) δ 2.70–3.60 (4 H, m, CH_2CH_2), 4.40 (2 H, s, CH_2), 6.50 (1 H, s, H_3), 7.93 (1 H, s, H_6). Anal. ($C_9H_{12}N_2O_4$) C, H, N] and **26** [mp 142–145 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.00–2.30 (6 H, m, $-CH_2CH_2CH_2$), 3.73 (2 H, br s, NH_2), 4.25 (3 H, br s, CH_2 and OH), 5.78 (1 H, s, H_3), 7.53 (1 H, s, H_6), 8.10 (1 H, br s, NH). Anal. ($C_{10}H_{14}N_2O_4$) C, H, N] were obtained respectively.

When the above procedure was repeated using *N*-(benzyloxycarbonyl)glycylglycine, **27**·HBr was obtained: mp 265–266 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 3.70 (2 H, br s, $CH_2NH_3^+$, collapses to a singlet on D_2O exchange), 3.88 (2 H, d, $COCH_2NH$, collapses to a singlet on D_2O exchange, $J_{CH_2NH} = 6$ Hz), 4.18 (2 H, d, CH_2NH, collapses to a singlet on D_2O exchange, $J_{CH_2NH} = 6$ Hz), 6.30 (1 H, s, H_3), 8.06 (5 H, br s, H_6, OH and $^+NH_3$), 8.66 and 8.80 (2 H, 2 t, 2NH). Anal. ($C_{10}H_{14}BrN_3O_5$) C, H, Br, N.

2-(Acetamidomethyl)-5-hydroxy-4*H*-pyran-4-one (28). A solution of 2-(acetamidomethyl)-5-(benzyloxy)-4*H*-pyran-4-one (5 g, 0.018 mol) in HOAc (120 mL) was saturated with HBr and stirred for 3 h. After evaporation, addition of *i*-PrOH, and reevaporation, the residue was slurried with $CHCl_3$ and filtered. Charcoaling in MeOH followed by evaporation and slurrying in $CHCl_3$ gave 3.02 g (91.7%) of **28**·H_2O: mp 212–214 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.90 (3 H, s, CH_3), 4.15 (2 H, d, CH_2, collapses to a singlet on D_2O exchange, $J_{CH_2NH} = 6$ Hz), 6.26 (1 H, s, H_3), 8.03 (1 H, s, H_6), 8.42 (1 H, triplet, NH, $J_{NHCH_2} = 6$ Hz), 9.23 (1 H, br s, OH). Anal. ($C_8H_{11}NO_5$) C, H, N.

Pharmacology. Skeletal muscle relaxant activity was evaluated in female Sprague-Dawley rats weighing 250–300 g or mongrel cats of either sex weighing 2.5–3.5 kg. Under ether or pentobarbital anesthesia, spinal section was performed at the midthoracic (T_{10}) level. At least 1 week later, the animals were suspended in a canvas sling, and a free hanging hindlimb was attached by a spring to a Grass FTO3C force displacement transducer connected to a Beckman Type R Dynograph. The flexor reflex was activated through platinum (Grass E-2) electrodes placed subcutaneously in the same hindlimb just anterior to the Achilles tendon. Rectangular pulses of 2 ms in duration, at a frequency of 100 Hz, were applied for 2 s at 5-min intervals with voltages sufficient to elicit a maximal tetanic ipsilateral flexor reflex. Test substances were dissolved in normal saline and administered intravenously or by oral gavage in cumulative doses at 15-min intervals. The cumulative dose which reduced the flexor reflex response by 70% or more (ED_{70}) from predrug control

responses was determined.

In 90–100% of Robidoux Swiss male mice weighing 18–22 g, intraperitoneal injections of 25 mg/kg of 3-mercaptopropionic acid induced tonic-extensor seizures within 3–7 min. Test substances were dissolved in saline and administered by the oral route 30 min prior to injection of the convulsant agent. The dose required to prevent tonic-extensor seizures in 50% of the animals (ED_{50}) was calculated using regression analysis and based upon at least three doses with ten mice per level.

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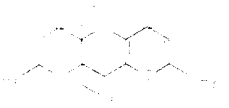
(26) Note Added in Proof: subsequent examination of the ^{13}C NMR spectrum of **10b** in Me_2SO-d_6 indicates that, in solution at least, it actually exists in the hemiketal form, i. Presumably **10a** also exists as this cyclized structure.



i = **10b**

No deduction concerning the stereochemistry could be made, but only one isomer is present, as judged from the spectrum.

Notes

Synthesis and Evaluation for Diuretic Activity of 1-Substituted 6-Chloro-5-sulfamylindolines

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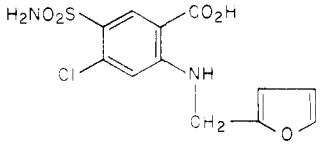
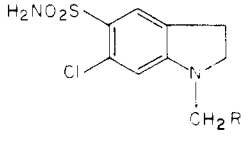
The synthesis of a series of 1-substituted 6-chloro-5-sulfamylindolines is described. In the Lipschitz test for diuretic activity, two of the compounds showed significant excretion of urine and sodium and were approximately equivalent in potency to chlorothiazide but with a later onset of activity.

Furosemide is a potent, high-ceiling diuretic, one of the most widely used in medicine today. We thought it would be of interest to synthesize and evaluate for diuretic activity a series of 1-substituted 6-chloro-5-sulfamylindolines (**7–9**) which can be regarded as nonacidic ring-closed



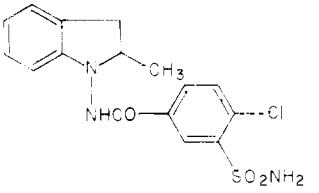
furosemide



7–9

analogues of furosemide. Recent reports of indolines[1,2] and isoindolines[3] with diuretic activity lent encouragement to this investigation.

One of the more promising indolines with diuretic properties demonstrated in clinical trials is indapamide.[2] As part of this present work on 1-substituted 6-chloro-5-sulfamylindolines, an analogue, **12**, structurally related to indapamide was also synthesized and tested.



indapamide

Chemistry. The starting material required for the

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