Kojic acid derivatives
I. Preparation and bromination of some 5-hydroxy-2-(R-thiomethyl)-4*H*-pyran-4-ones

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By the reaction of chlorokojic acid with some sodium thiolates, 5-hydroxy-2-(R-thiomethyl)-4*H*-pyran-4-ones were prepared. Bromination of these compounds using *N*-bromosuccinimide afforded corresponding 6-bromo derivatives. The structure of the prepared pyranones was proved by IR, ^{1}H NMR, ^{13}C NMR, and mass spectral data. Their antimicrobial activity was also determined.

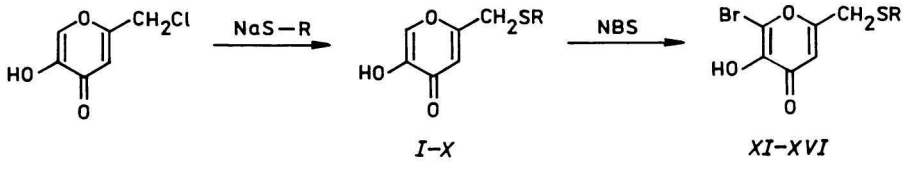
Kojic acid — 5-hydroxy-2-(hydroxymethyl)-4*H*-pyran-4-one, arising from saccharidic sources by the action of various aerobic microorganisms, is a potentially important synthetic intermediate of many 4*H*-pyran-4-one derivatives [1]. This compound was also studied from the point of view of its application in pharmacy and cosmetic [2—7], as it is known to exhibit some antimicrobial activity [8], attributed at first to its ability to chelate some metal cations [9]. Further studies showed [10] that this chelation is not necessary for exhibition of bacteriostatic activity and that for an increase of effect, electron-releasing substituent at the position 7 and the withdrawing of electrons from hydroxyl group at the position 5 of γ-pyrone ring should have decisive influence [11].

In this paper, we have focused our attention on the preparation of such derivatives where electron donor is represented by sulfur attached in the form of sulfide. Our aim was to examine the influence of this substitution on antimicrobial efficiency. Some derivatives were synthesized as intermediates for the preparation of compounds having tenside properties.

By the reaction of chlorokojic acid [12] with selected sodium thiolate according to the analogy from the literature [13], corresponding sulfides *I*—*X* (Scheme 1) were prepared in 30—97 % yields. This simple nucleophilic substitution required specification only in the case of preparation of individual sodium thiolates (see Experimental). In the next step, the prepared compounds were brominated by using *N*-bromosuccinimide [14]. This bromination takes place at C-6 atom (compounds *XI*—*XVI*, Scheme 1), but only in that case, if hydroxyl group at C-5 position is unprotected. In the case of acylated or benzoylated derivatives, bromination does not take place [15]. Likewise, we were unsuccess-

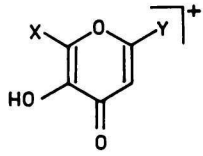
ful to prepare corresponding 6-bromo derivatives starting from compounds *II*, *VII*, *VIII*, and *IX*.

The results of elemental analysis, yields and melting points of the prepared compounds (all crystalline products) are given in Table 1.



Scheme 1

In the mass spectra (70 eV) of all prepared compounds, molecular ions $M^{+\bullet}$ were observed. Their intensity (I_r) was considerably dependent on the character of substituents at the positions 6 and 7. In the case of unbrominated derivatives *I*—*X*, the I_r was in the range of 12—29 %, only compounds *I*, *VI*, and *X* exhibited considerably higher values (65 % for *I*, 77 % for *VI*, and 40 % for *X*). Brominated compounds *XI*—*XVI* showed substantially lower intensity of molecular ions (I_r = 3—11 %). Further significant peaks in the spectra of prepared compounds corresponding to the ions A and B were formed by the fragmentation of CH_2—S bond.



	X	Y
A	H	CH_2
B	Br	CH_2
C	H	CH_2S
D	Br	CH_2S

Their intensity was also dependent on the character of substituent. In the case of compounds *I* and *V*, these ions corresponded to the base peaks of spectrum (I_r = 100 %). Compounds *I*—*V* and *XII*—*XIV* exhibited also peaks belonging to the ions C and D, formed by the fragmentation of S—R bond (I_r = = 11—26 %). Moreover, in the spectra of brominated compounds *XI*—*XVI*, the peaks corresponding to the ions formed by the detachment of bromine atom from molecule (I_r = 22—28 %, for compound *XIV* up to 83 %) were observed.

In the IR spectra of the prepared compounds, strong absorption bands in the region of $\tilde{\nu}$ = 1639—1648 cm^{-1} corresponding to the vibrations ν(C=O) were observed which together with two intensive bands in the region of $\tilde{\nu}$ = = 1604—1613 cm^{-1} and $\tilde{\nu}$ = 1575—1583 cm^{-1} corresponding to the vibrations ν(C=C) are characteristic of γ-pyrone skeleton of kojic acid. The bands at

Table 1

Characterization of the prepared compounds

Compound	R	Formula	M_r	w_i(calc.)/% w_i(found)/%					Yield %	M.p. °C
				C	H	S	N	Br		
I	2-Hydroxyethyl	$C_8H_{10}O_4S$	202.14	47.53	4.94	15.86	—	—	43	103—104
				47.48	5.02	15.91				
II	Carboxymethyl	$C_8H_8O_5S$	216.14	44.45	3.70	14.83	—	—	45	157—158
				44.51	3.76	14.90				
III	Heptyl	$C_{13}H_{20}O_3S$	256.19	60.94	7.80	12.52	—	—	80	81—83
				60.86	7.87	12.58				
IV	Octyl	$C_{14}H_{22}O_3S$	270.20	62.23	8.14	11.87	—	—	70	74—75
				62.30	8.19	11.92				
V	Dodecyl	$C_{18}H_{30}O_3S$	326.24	66.26	9.19	9.83	—	—	62	85—86
				66.20	9.25	9.89				
VI	Phenyl	$C_{12}H_{10}O_3S$	234.18	61.54	4.27	13.69	—	—	66	93—94
				61.58	4.30	13.63				
VII	2-Aminophenyl	$C_{12}H_{11}NO_3S$	249.28	57.81	4.42	12.86	5.61	—	34	167—168
				57.74	4.46	12.91	5.64			
VIII	2-Benzimidazolyl	$C_{13}H_{10}N_2O_3S$	274.19	56.94	3.65	11.69	10.21	—	97	104—105
				56.99	3.69	11.61	10.28			
IX	4,6-Dimethyl-2-pyrimidinyl	$C_{12}H_{12}N_2O_3S$	264.18	54.55	4.54	12.13	10.60	—	55	132—133
				54.51	4.59	12.06	10.65			

Table 1 (Continued)

Compound	R	Formula	M_r	w_i(calc.)/% / w_i(found)/%					Yield %	M.p. °C
				C	H	S	N	Br		
X	4,6-Diamino-2-pyrimidinyl	$C_{10}H_{10}N_4O_3S$	266.16	45.08	3.76	12.06	21.04	—	65	245—246
				45.14	3.80	12.00	21.07			
XI	2-Hydroxyethyl	$C_8H_9BrO_4S$	281.04	34.19	3.20	11.40	—	28.43	16	104—105
				34.14	3.22	11.37		28.38		
XII	Heptyl	$C_{13}H_{19}BrO_3S$	335.09	46.59	5.67	9.57	—	23.84	60	64—65
				46.56	5.69	9.53		23.79		
XIII	Octyl	$C_{14}H_{21}BrO_3S$	349.10	48.16	6.02	9.18	—	22.89	81	76—78
				48.19	6.06	9.16		22.86		
XIV	Dodecyl	$C_{18}H_{29}BrO_3S$	405.14	53.35	7.15	7.91	—	19.72	75	89—90
				53.32	7.17	7.87		19.68		
XV	Phenyl	$C_{12}H_9BrO_3S$	313.08	46.03	2.87	10.24	—	25.52	29	159—160
				46.02	2.89	10.20		25.47		
XVI	4,6-Dimethyl-2-pyrimidinyl	$C_{12}H_{11}BrN_2O_3S$	343.08	42.00	3.20	9.34	8.16	23.29	88	176—177
				42.03	3.22	9.33	8.17	23.25		

$\tilde{\nu}$ = 3230—3244 cm^{-1} corresponding to the associated OH groups and the bands at $\tilde{\nu}(\nu_{as}(C—O—C))$ = 1218—1231 cm^{-1} are also characteristic. Stretching vibrations of the sulfidic C—S bond showed weak absorption bands in the region of $\tilde{\nu}(\nu_s)$ = 629—635 cm^{-1} and $\tilde{\nu}(\nu_{as})$ = 720—724 cm^{-1}

The data of ^1H NMR and ^{13}C NMR spectra confirming the structure of selected compounds are given in Experimental.

The results of antimicrobial activity testing showed relatively low activity of this type of kojic acid derivatives. In the case of unbrominated derivatives *I*—*X*, the minimum inhibitory concentration (MIC) was about 1000 ppm for gram-negative and gram-positive bacteria, for fungi MIC ranged within 100—1000 ppm. Only compound *IV* exhibited 10 ppm for *Trichophyton terrestre*. In the case of brominated derivatives, only compounds *XI* and *XII* showed higher efficiency against *Staphylococcus aureus* (MIC = 100 ppm). On the other hand, efficiency against fungi *Trichophyton terrestre* and *Microsporum gypseum* was tenfold lower (MIC = 100 resp. 1000 ppm) than in the case of unbrominated derivatives. For gram-negative bacteria (*Escherichia coli*, *Bacillus subtilis*, *Salmonella typhimurium*, *Shigella flexneri*), substitution had no influence on the antimicrobial activity — MIC was 1000 ppm for all compounds.

Experimental

Chlorokojic acid was prepared by the chlorination of kojic acid using thionyl chloride [12]. The used thiols were commercial products (Lachema, Brno; Fluka, Buchs; Merck, Darmstadt).

The melting points were determined on a Kofler hot-stage. IR spectra (in KBr pellets) were obtained on a Perkin—Elmer G-983 instrument. Mass spectra (U = 70 eV) were measured on a JMS-100D spectrometer at an emission current of 300 μA, applying direct sample-introduction technique. ^1H NMR spectra ($CDCl_3$) were registered on a FT-NMR Bruker AM-300 spectrometer (300.13 MHz) using TMS as an internal standard. ^{13}C NMR spectra (75.46 MHz) were measured at the same conditions. The data of elemental analysis were obtained on a Perkin—Elmer 240 analyzer.

MIC was determined by using qualitative suspension method on solid cultivation media (cultivation medium No. 2, Imuna, for bacteria and Sabouraud's medium for fungi and yeasts). The solutions of examined compounds in ethanol (96 %) were used for testing. The growth of microorganisms at different concentrations of tested compound was evaluated. The used microorganisms were from the Czechoslovak state collection of species cultures.

5-Hydroxy-2-(2-hydroxyethylthiomethyl)-4H-pyran-4-one (I)

To a solution of sodium ethanolate (0.05 mol) in ethanol (60 cm^3), 2-mercaptoethanol (0.05 mol) was added under intensive stirring and the mixture was heated under reflux for

1 h. After cooling to 10 °C, chlorokojic acid (0.05 mol) was added and the mixture was stirred at this temperature for 1 h. Then, heating under reflux for 3 h followed. Still hot solution was filtered, solvent evaporated under diminished pressure and the product was recrystallized from ethyl acetate.

Analogical procedure was used for the preparation of compounds *VII*—*X*.

5-Hydroxy-2-(carboxymethylthiomethyl)-4H-pyran-4-one (II)

Thioglycolic acid (0.05 mol) was added to the sodium ethanolate (0.1 mol) in ethanol (60 cm^3). The mixture was stirred at room temperature for 1 h, then chlorokojic acid (0.05 mol) was added and the mixture was heated under reflux for 4 h. After cooling, glacial acetic acid (0.05 mol) was added and the solvent was distilled off under diminished pressure. After an addition of water (50 cm^3), the product was extracted into ethyl acetate, extract dried over Na_2SO_4, solvent evaporated to the one third of its volume. After cooling, crystalline product was obtained.

5-Hydroxy-2-(R-thiomethyl)-4H-pyran-4-ones III—VI

To the suspension of sodium (0.05 mol) in toluene (50 cm^3) at 70 °C, a solution of corresponding thiol (0.05 mol) in toluene (50 cm^3) was added under stirring in the course of 15 min. The mixture was heated under reflux for 2 h, then cooled to 10—15 °C. Chlorokojic acid (0.05 mol) was added and the mixture was heated under reflux for additional 3 h. After cooling, cold water (100 cm^3) and ether (100 cm^3) was added and the mixture was left to stand in separatory funnel overnight (in the case of derivatives with long alkyl chain, separation is very slow owing to the formation of emulsions). Organic layer was separated, dried over Na_2SO_4, filtered and the solvents were evaporated under diminished pressure. Crude products *III* and *IV* were recrystallized from the mixture heptane—ether ($\varphi_r = 1$ 1), compounds *V* and *VI* were recrystallized from hexane.

For compound *V* ^{1}H NMR spectrum ($CDCl_3$), δ: 0.88 (t, 3H, CH_3), 1.15—1.40 (m, 6H, $SCH_2CH_2\underline{CH_2}$, $C\underline{H_2}C\underline{H_2}CH_3$), 1.26 (s, 12H, $(CH_2)_6$), 1.52—1.62 (m, 2H, $SCH_2\underline{CH_2}$), 2.53 (t, 2H, $S\underline{CH_2}CH_2$), 3.52 (s, 2H, $=\underset{|}{C}-CH_2-S-$), 6.45 (s, 1H, H-3), 7.86 (s, 1H, H-6). ^{13}C NMR spectrum ($CDCl_3$), δ: 14.11 (CH_3), 22.68—33.76 (12C, CH_2), 111.06 (C-3), 138.31 (C-6), 145.41 (C-5), 166.38 (C-2), 173.94 (C-4).

For compound *VI* ^{1}H NMR spectrum ($CDCl_3$), δ: 3.86 (s, 2H, CH_2S), 6.28 (s, 1H, H-3), 7.24—7.38 (m, 5H, C_6H_5), 7.81 (s, 1H, H-6). ^{13}C NMR spectrum ($CDCl_3$), δ: 37.34 (CH_2S), 111.50 (C-3), 128.09 (C-4′), 129.28 (C-2′, C-6′), 131.80 (C-3′, C-5′), 133.10 (C-1′), 138.41 (C-6), 145.45 (C-5), 165.34 (C-2), 173.83 (C-4).

6-Bromo-5-hydroxy-2-(R-thiomethyl)-4H-pyran-4-ones XI—XVI

To the solution of corresponding 5-hydroxy-2-(R-thiomethyl)-4*H*-pyran-4-one (5 mmol) in dry benzene (50 cm^3), *N*-bromosuccinimide (5.5 mmol) was added. Reaction

mixture was heated under reflux for 1 h and then it was left overnight at room temperature. Separated succinimide was sucked off, benzene solution was washed with water (50 cm^3) and dried over Na_2SO_4. After evaporation of solvent under diminished pressure, crude product was crystallized from hexane.

For compound *XIV* ^{1}H NMR spectrum ($CDCl_3$), δ: 0.88 (t, 3H, CH_3), 1.15—1.40 (m, 6H, $SCH_2CH_2\underline{CH_2}$, $C\underline{H_2}C\underline{H_2}CH_3$), 1.26 (s, 12H, $(CH_2)_6$), 1.52—1.62 (m, 2H, $SCH_2\underline{CH_2}$), 2.56 (t, 2H, $SC\underline{H_2}CH_2$), 3.53 (s, 2H, $=\underset{|}{C}—CH_2—S—$), 6.42 (s, 1H, H-3). ^{13}C NMR spectrum ($CDCl_3$), δ: 14.12 (CH_3), 22.68—33.48 (12C, CH_2), 110.53 (C-3), 139.40 (C-6), 144.01 (C-5), 167.22 (C-2), 172.35 (C-4).

For compound *XV* ^{1}H NMR spectrum ($CDCl_3$), δ: 3.86 (s, 2H, CH_2S), 6.22 (s, 1H, H-3), 7.26—7.44 (m, 5H, C_6H_5). ^{13}C NMR spectrum ($CDCl_3$), δ: 37.18 (CH_2S), 110.95 (C-3), 128.30 (C-4′), 129.36 (C-2′, C-6′), 132.05 (C-3′, C-5′), 133.12 (C-1′), 141.18 (C-6), 144.03 (C-5), 166.13 (C-2), 172.19 (C-4).

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