SOME NEW REACTIONS AND DERIVATIVES OF KOJIC ACID[1]

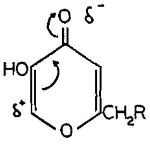
BY ANDREW BEÉLIK[2] AND C. B. PURVES

ABSTRACT

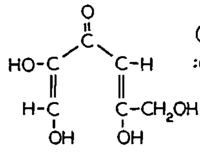
The stability of the ring structure in kojic acid toward various reagents was investigated. Cleavage to an open-chain, dienol derivative in N sodium hydroxide at 25°, or fragmentation of the structure, was very slow. The benzoyl group in the fifth (phenolic) position of dibenzoylkojic acid was removed by hydroxylamine hydrochloride in pyridine so selectively that the method was of value in synthesizing certain new derivatives. Although catalytic hydrogenation readily reduced the pyrone ring in kojic acid to undefined substances, zinc dust in glacial acetic acid reduced the hydroxymethyl group in the dibenzoate to a methyl group and yielded benzoylallomaltol. The ring in dibenzoylkojic acid was apparently opened, with retention of both benzoyl groups, by semicarbazide hydrochloride and pyridine to yield two isomeric "disemicarbazones", $C_{22}H_{22}N_6O_7$, decomp., 215° and 172–173°, respectively. The higher-melting isomer when boiled with dilute acid gave a compound $C_{21}H_{15}N_3O_5$, m.p. 244°, which was apparently cyclic; the same isomer with nitrous acid yielded two isomeric, apparently open-chain "monosemicarbazones", $C_{21}H_{19}N_3O_7$, decomp., 215° and 178–179°, respectively. The structures of these five compounds were not determined. The following substances were thought to be new: sodium kojate, a white powder, and its crystalline tetrahydrate; 5-hydroxy-2-(methoxymethyl)-γ-pyrone, m.p. 75–76°, and its crystalline aluminum salt, decomp. 270–271°; molecular addition compound of dibenzoylkojic acid and benzoic acid, m.p. 120–121°; 2-(acetoxymethyl)-5-benzoxy-γ-pyrone, m.p. 144°; 2-(benzoxymethyl)-5-hydroxy-γ-pyrone, m.p. 180–181°; and 2-(benzoxymethyl)-5-methoxy-γ-pyrone, m.p. 110–111°. A more convenient synthesis was discovered for 5-benzoxy-2-(hydroxymethyl)-γ-pyrone, and the published melting point was revised from 136° to 143–144°; the melting point of 5-benzoxy-2-(triphenylmethoxymethyl)-γ-pyrone was revised from 206–208° to 213–214°.

INTRODUCTION

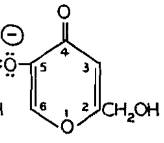
Kojic acid, (5-hydroxy-2-(hydroxymethyl)-γ-pyrone) (I), is a crystalline substance which can be produced in good yield by the action of many molds of the *Aspergillus* family on a wide range of carbohydrates. This biological synthesis has been studied extensively because it is the simplest example of the conversion of a sugar to a γ-pyrone. The possibility of utilizing industrial carbohydrate by-products by converting them to kojic acid has also stimulated interest in the chemical properties of this compound. Barham and Smits



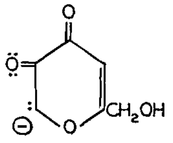
I, R = OH
II, R = H
III, R = Cl



Ia



Ib



Ic

---

[1] *Manuscript received March 29, 1955.*
Contribution from the Division of Industrial and Cellulose Chemistry, McGill University, and the Wood Chemistry Division, Pulp and Paper Research Institute of Canada, Montreal, Que. Abstracted from a Ph.D. thesis submitted by A. B. to the University in September, 1954.

[2] *Present address: Department of Chemistry, Royal Military College of Canada, Kingston, Ontario.*

(4) have summarized the earlier literature, and a new review is to appear elsewhere (6).

Practically all the known derivatives of kojic acid were formed by reactions involving the two hydroxyl groups, by nuclear substitutions at position 6, or by the replacement of the ring oxygen atom with the nitrogen of ammonia or amines to give γ-pyridones (6, 11, 12). A few γ-pyrones other than kojic acid, however, were cleaved at the ring oxygen atom by cold dilute aqueous alkalies to yield open-chain derivatives. With the possible exception of Haitinger and Lieben's "xanthochelidonic acid" (10), the dienolic compounds initially produced underwent further changes to more stable ketonic forms (19). Acidification (10), or heating with dilute acid (19), usually regenerated the original γ-pyrone. Willstätter and Pummerer (20) prepared stable derivatives of the dienolic cleavage product of γ-pyrone itself by methylation and benzoylation in the alkaline reaction mixture. The similar open-chain form (I*a*) was assumed as the final intermediate in the biosynthesis of kojic acid (6), but the only experimental evidence for its existence was the fact that kojic acid in warm dilute alkali resembled other α-carbonyl dienol structures in reducing Tillman's reagent (2,6-dichlorophenol-indophenol) (18). When the alkali was more concentrated, or was heated, the pyrone ring was fragmented; thus Yabuta (22, 23), and more recently Arnstein and Bentley (3), found that dimethylkojic acid was degraded to formic acid, methoxyacetic acid, and methoxyacetone by boiling aqueous barium hydroxide. The present research had the object of studying the action of bases on kojic acid under milder conditions, and of isolating open-chain derivatives in an unfragmented state.

One-gram samples of kojic acid were accordingly dissolved in approximately 1.2 *N* sodium hydroxide and the clear yellow solutions were acidified with 20% sulphuric acid after various times. Table I, column 4, records the amount of kojic acid which crystallized when the acidified liquor was kept at −3°; in some experiments the mother liquors were concentrated to recover a second fraction, and in others 60% of the volume was distilled at atmospheric pressure in order to estimate any formic acid produced. Even after 32 hr. in an excess of the alkali, more than 80% of the kojic acid could be recovered as such,

TABLE I

STABILITY OF KOJIC ACID IN APPROXIMATELY 1.2 *N* AQUEOUS SODIUM HYDROXIDE AT 25°

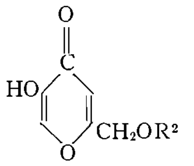
			Kojic acid recovered			
NaOH, mM.[a]	Water, ml.	Time, hr.	Fract. 1, gm.	Fract. 2, gm.	Total, %	Formic acid, mM. %[a]
2	11.3	1.2	0.76	0.13	89	—
2	11.3	3.0	0.73	0.15	88	—
2	11.3	13.5	0.77	—	—	1.0
2	11.3	32	0.74	—	—	2.5
3	16.9	1.0	0.66	0.19	85	—
3	16.9	32	0.74	—	—	3.2
5	28.2	1.0	0.56	0.25	81	—

[a] *Per mM. of kojic acid, 1 gm. or 7.06 mM. of which was used in all cases.*

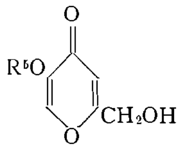
and only a few per cent had been decomposed to formic acid. Kojic acid was therefore either nearly stable in these conditions, or its hypothetical open-chain enolic form (I*a*) recyclized almost quantitatively when the solution was acidified.

In attempts to methylate or benzoylate any enolic compound formed, dimethyl sulphate or benzoyl chloride was added to solutions of kojic acid in aqueous alkali. The methylation was carried out with a molar ratio of 1:6:3.7 for kojic acid, potassium hydroxide, and dimethyl sulphate, respectively, but the only product, obtained in low yield, was a new, crystalline monomethyl ether of kojic acid itself. This ether imparted a deep wine color to ferric chloride solution and therefore contained a free phenolic hydroxyl group; methylation with diazomethane gave the known 5-methoxy-2-(methoxymethyl)-γ-pyrone. Hence the new monomethyl ether was 5-hydroxy-2-(methoxymethyl)-γ-pyrone (IV). In the process of purification, IV reacted with the alumina in a chromatograph column to give a pale yellow, crystalline derivative in which three moles were substituted by one atom of aluminum; that is, the derivative had the composition of the aluminum phenoxide. The isomeric monomethyl ether, 2-(hydroxymethyl)-5-methoxy-γ-pyrone (VIII), was obtained in a crude yield of 72% by Campbell and his co-workers (8) from kojic acid, potassium hydroxide, and dimethyl sulphate in a 1:1:1 molar ratio. Heyns and Vogelsang (11) prepared the dimethyl ether from the same reagents, using a molar ratio of 1:8:9.

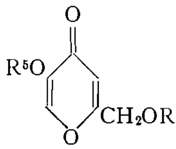
Two methods were used for the benzoylations, differing mainly in the excess of sodium hydroxide present throughout the reaction. When this excess was considerable, the products were the known, crystalline dibenzoylkojic acid (5-benzoxy-2-(benzoxymethyl)-γ-pyrone) (22, 23) and a new, crystalline addition compound, m.p. 120–121°, between equimolecular amounts of this dibenzoate and benzoic acid. The infrared spectrum of a benzene solution of the adduct was identical with that given by an equimolecular mixture of its components, a Perkin-Elmer Model 21 spectrophotometer fitted with a rock salt prism being used for the determination. This addition compound could be recrystallized without change from hydrocarbon solvents, but dissociated into its constituents when dissolved in polar liquids. When the excess of alkali used in the benzoylation was small, the product consisted of the above adduct, together with a monobenzoyl derivative of kojic acid which melted at 142–143°. This melting point was 7° higher than a value reported by Yabuta for a mono-



IV R^2 = methyl
V R^2 = benzoyl
VI R^2 = acetyl
VII R^2 = trityl



VIII R^5 = methyl
IX R^5 = benzoyl
X R^5 = sodium



XI R^2 = trityl; R^5 = benzoyl
XII R^2 = benzoyl; R^5 = methyl
XIII R^2 = acetyl; R^5 = benzoyl

benzoate prepared from kojic acid and benzoyl chloride in ether (22, 23), or from 5-benzoxy-2-(triphenylmethoxymethyl)-γ-pyrone (XI) by detritylation (25). The latter unequivocal synthesis was repeated, the proper melting point of the resulting 5-benzoxy-2-(hydroxymethyl)-γ-pyrone (IX) was found to be 142–143°, and identity with the monobenzoate under discussion was proved.

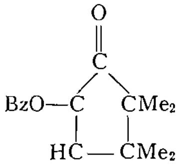
Other attempts to isolate a derivative of I*a* involved the complete evaporation of aqueous solutions of kojic acid containing one or two molecular equivalents of sodium hydroxide. The product was the new sodium kojate, which occurred either as a crystalline tetrahydrate or in the anhydrous condition. This "salt" was presumably the 5-sodio derivative (X). The tetrahydrate reacted with benzoyl chloride in dry benzene to give the 5-monobenzoate (IX); the yield of the latter rose to 79.5% when anhydrous sodium kojate was used. These observations, together with the results of the methylations and benzoylations, seemed to indicate the absence of an open-chain form like I*a* from solutions of kojic acid in dilute aqueous alkali.

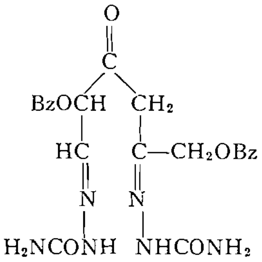
As was expected from the inert nature of the carbonyl group, no derivatives could be isolated when kojic acid was treated with hydroxylamine hydrochloride or with semicarbazide hydrochloride. The customary procedures for oximation, however, produced from dibenzoylkojic acid in up to 90% yield a crystalline substance which proved to be a new monobenzoate and not an oxime. Benzoylation in dry pyridine gave back the dibenzoate, and the presence of a free phenolic function in the monobenzoate was indicated by the wine color it produced with ferric chloride. Methylation with diazomethane yielded another new crystalline substance which was identified as 2-(benzoxymethyl)-5-methoxy-γ-pyrone (XII) by a synthesis from VIII and benzoyl chloride. The monobenzoate was therefore the 2-derivative (V).

The selective deacylation displayed by hydroxylamine in the above reaction was also illustrated by the smooth debenzoylation of the 5-benzoxy derivatives of 2-acetoxymethyl- (XIII) and 2-(triphenylmethoxymethyl)-γ-pyrone (XI), and of benzoylallomaltol to the corresponding 5-hydroxy derivatives (VI, VII, and II). The method should thus be useful for preparing derivatives of kojic acid monosubstituted in the generally less reactive 2-position, especially when a substituent such as acetyl or trityl offers but little resistance to conventional methods of hydrolysis. Although the cleavage of esters by hydroxylamine in the presence of a strong base is frequently used for the preparation of substituted hydroxamic acids (26), the method has not hitherto been employed for selective deacylations. Ingold and Shoppee (15), however, incidentally observed that their oximation of 2-benzoxy-4,4,5,5-tetramethyl-2-cyclopenten-1-one (XIV) was accompanied by debenzoylation, although the benzoyl group in the analogous saturated ketone was unaffected under identical conditions. The results support the view that enolic and phenolic ester groups adjacent to a carbonyl group are cleaved with particular ease by hydroxylamine. It therefore seems probable that the same method might prove useful in other compounds containing the same grouping; for example, in the selective deacylation of flavonols at position 3.

Another selective debenzoylation, this time involving the group in the second instead of the fifth position of dibenzoylkojic acid, was brought about by reduction with zinc dust in glacial acetic acid. The products included nearly one mole of benzoic acid and a crystalline substance which was proved to be the benzoate of allomaltol (II) (5-benzoxy-2-methyl-γ-pyrone). This proof consisted of repeating Yabuta's reduction (24) of 2-(chloromethyl)-5-hydroxy-γ-pyrone (III) to the 2-methyl derivative (II) and benzoylating the latter. The primary alcohol unit in dibenzoylkojic acid thus resembled that in benzyl ethers in its tendency to undergo reductive fission. In accord with this inference, the dibenzoate yielded 0.6 mole of benzoic acid when hydrogenated at room temperature and atmospheric pressure over a platinum catalyst. This hydrogenation probably involved hydrogenolysis, since hydrolysis was not likely in the glacial acetic acid used as a solvent. The other product of the hydrogenation was a sirup which was not studied in detail because numerous earlier catalytic hydrogenations of kojic acid, its ethers and esters, gave sirups of indefinite composition (6). Ingold and Shoppee (15) observed that the enolic benzoyl group in (XIV) was unaffected by zinc or sodium amalgam in glacial acetic acid, while the double bond was readily reduced (with retention of the benzoyl group) by hydrogenation.

Dibenzoylkojic acid failed to form a benzoylhydrazone and a 2,4-dinitrophenylhydrazone, but condensation occurred with semicarbazide hydrochloride in boiling ethanol–pyridine. The product consisted of two white, neutral, microcrystalline isomers, but the one with the lower melting point was formed in too small yield to be examined in detail. The molecular formula of these isomers, $C_{22}H_{22}N_6O_7$, corresponded to disemicarbazones of an open-chain form of dibenzoylkojic acid, and structures such as XV were tentatively assigned to them. The higher-melting isomer was readily saponified by cold dilute alkali, but the only product identified was benzoic acid in nearly quantitative yield. These structures were unusual, for the initial open-chain products from other γ-pyrones and hydrazines invariably lost one additional molecule of water to give cyclic end products. Bedekar and co-workers (5) represented these end products as pyridones, but Ainsworth and Jones (1) recently found very good evidence in favor of pyrazole structures. No carboxylic acid derived from a pyridone or pyrazole, however, could be recovered when the higher-melting isomer was oxidized with potassium permanganate, only benzoic and benzoyl-


XIV


XV

glycolic acids being identified. When the same isomer was boiled in 10% hydrochloric acid, the yellow-white, microcrystalline substance which formed in small yield had the molecular formula $C_{21}H_{15}N_3O_5$, which corresponded to the loss of one semicarbazide unit and two molecules of water. Cyclization probably occurred in this case, but the point could not be definitely proved owing to lack of material.

The higher-melting "disemicarbazone" lost one semicarbazide group on treatment with nitrous acid (9, 21), and two neutral, microcrystalline "monosemicarbazones" resulted. The lower-melting, or "β", form was the main product (69%), and the "α-isomer" was isolated only when a larger quantity of nitrous acid was employed. Since an excess of nitrous acid did not convert the β- to the "α-monosemicarbazone" the two were perhaps position isomers. To judge from the composition of these two products, $C_{21}H_{19}N_3O_7$, both were open-chain derivatives and no cyclization occurred during the treatment with nitrous acid. No carboxylic acids other than benzoic and benzoylglycolic could be isolated when the β-isomer was oxidized with aqueous potassium permanganate. An attempt to regenerate the "disemicarbazone" from the "β-monosemicarbazone" by treating the latter with semicarbazide hydrochloride and pyridine in boiling methanol failed, the starting material being recovered unchanged. Hydroxylamine hydrochloride likewise had no effect. These results were surprising, because the presence of a carbonyl group created by the fission of a semicarbazone unit could reasonably have been assumed (21); the negative outcome with hydroxylamine also suggested that neither of the two benzoyl groups was of the phenolic type present in dibenzoylkojic acid. At this point, attempts to elucidate the structures of the "monosemicarbazones" had to be abandoned.

The failure of *N* sodium hydroxide to open the ring of kojic acid stood in sharp contrast to the apparent cleavage of the ring in the dibenzoate by semicarbazide, a much weaker base. Cleavage of γ-pyrone rings was presumably initiated by a nucleophilic reagent at position 6, where the drift of electrons toward the carbonyl group would create an electrophilic center. This might happen in the case of dibenzoylkojic acid, as indicated in I, when semicarbazide was the attacking nucleophile (14). In sodium hydroxide, kojic acid would be present as the kojate anion (I*b*), and an important contribution to the resonance hybrid of this anion could be expected from the diketo form (I*c*) with the full negative charge located at position 6. This nucleophilic center would face the nucleophilic hydroxyl ion, and no cleavage would result. If this explanation was correct, the production of open-chain forms of kojic acid by basic cleavage required either the use of very weak bases or the blocking of the hydroxyl group in position 5 by a substituent stable to alkali.

EXPERIMENTAL

All melting points were corrected. All of the nitrogen analyses, and two carbon and hydrogen analyses marked by an asterisk, were by Schwarzkopf Microanalytical Laboratory, Woodside, N.Y., U.S.A. Reactants were always weighed to the nearest 10 mgm.

5-Hydroxy-2-methoxymethyl-γ-pyrone (IV) and Its Aluminum "Salt"

Dimethyl sulphate, 47 gm. (0.373 mole), was slowly added over a period of three hours and at room temperature to a stirred solution of 15 gm. (0.106 mole) of kojic acid and 37.5 gm. (0.6 mole) of potassium hydroxide in 315 ml. of water. The mixture slowly warmed to 37°, but was then kept below 30°. The alkaline liquor, having been concentrated at 50° *in vacuo* to 250 ml., was extracted continuously with benzene to remove any dimethylkojic acid, but the yellow extract yielded only traces of an oil. The aqueous liquor was then acidified to pH 2 with 50% sulphuric acid and was re-extracted continuously with benzene for 21 hr. This dark red extract was treated with activated carbon, concentrated to small volume, and diluted with ligroin. The red oil which separated solidified on chilling to a yellow-brown substance, 6.85 gm. melting between 55° and 66°. Crystallization from ethyl acetate left 1.85 gm. (11.2%), m.p. 75–76°, unchanged by recrystallization from toluene–hexane. Found: C, 53.7, 53.8; H, 5.3, 5.2; OCH_3, 20.4, 20.4%. Calc. for $C_6H_5O_3(OCH_3)$: C, 53.9; H, 5.1; OCH_3, 19.9%.

The combined mother liquors from the above crystallizations were evaporated, and a concentrated solution of the residue in benzene was applied to a short column of alumina. Elution with benzene, evaporation of the eluates, and recrystallization of the orange products from ethanol gave 0.71 gm. (4%) of pale yellow crystals melting with decomposition at 270–271°. A qualitative test demonstrated the presence of aluminum. Found: Ash, 10.4; Al, 5.5; OCH_3, 18.6%. Calc. for $Al\,[C_6H_4O_3(OCH_3)]_3$: Ash, 10.4; Al, 5.5; OCH_3, 18.9%.

A solution of 0.15 gm. of these crystals in water was adjusted to pH 2 with dilute sulphuric acid and evaporated cautiously to a sirup. Extraction of this sirup with toluene yielded 0.09 gm. (63%) of 5-hydroxy-2-(methoxymethyl)-γ-pyrone with the proper melting point, undepressed by admixture with an authentic sample.

A 0.5 gm. sample of the monomethyl ether was kept dissolved in 20 ml. of dry benzene containing about 0.56 gm. of diazomethane (2) for 12 hr. at room temperature. Evaporation of this solution, and crystallization of the yellow residue from benzene–hexane, yielded 0.33 gm. (60.5%) of white needles melting at 89–90°, undepressed by admixture with an authentic sample of dimethylkojic acid (11).

Sodium Kojate and Its Crystalline Tetrahydrate

A solution of 3.0 gm. (21 mM.) of kojic acid and 0.88 gm. (21 mM.) of sodium hydroxide in 15 ml. of water was evaporated to dryness *in vacuo* at 40°. The residual solid was recrystallized twice from 5 ml. of hot water, and the white crystals were dried in air at 25°. Yield, 2.62 gm. or 53%. Found: Na, 10.3; loss of weight at 110°, 30.3, 30.2; at 25° over phosphorus pentoxide, 29.7%. Calc. for $C_6H_5O_4Na.4H_2O$: Na, 9.8; water, 30.5%.

The water of crystallization was determined by drying samples either for about six hours at 110° or for about 18 hr. *in vacuo* over phosphorus pentoxide at 25°. Anhydrous sodium kojate was a white powder when prepared by the latter method, and a tan powder by the former.

A New Synthesis of 5-Benzoxy-2-(hydroxymethyl)-γ-pyrone

Crude sodium kojate was prepared from 2 gm. (14 mM.) of kojic acid as just described, and was rendered anhydrous by drying *in vacuo* over phosphorus pentoxide. The product was shaken in the original tightly-stoppered flask with 50 ml. of dry benzene and 2.5 gm. (18 mM.) of benzoyl chloride at room temperature for 26 hr. It was necessary to interrupt the shaking to loosen solid material which adhered initially to the walls of the flask. After adding 30 ml. of benzene, the white suspension was heated to boiling and was filtered while hot. The residual solid was re-extracted with hot benzene, then with water, and again with benzene. The combined benzene solutions, when dried and evaporated, deposited 2.53 gm. of white needles melting at 143–144°, together with a second fraction which was recrystallized from aqueous acetone to give 0.22 gm. with the proper melting point. Total yield, 79.5%. A mixed melting point with authentic 5-benzoxy-2-(hydroxymethyl)-γ-pyrone (see below) was not depressed.

The authentic sample was prepared from 5-benzoxy-2-(triphenylmethoxymethyl)-γ-pyrone, (XI), which when recrystallized from aqueous dioxane melted at 213–214°, and not at 206–208° as recorded by Yabuta and Anno (25). The removal of the trityl group with boiling 80% acetic acid, followed by the separation of triphenylcarbinol, left a product which was crystallized from benzene. Yield, 55%, and melting point 140–141°. Further recrystallizations from benzene raised the melting point to 142–143°, the value quoted by Yabuta being 135–136°.

Dibenzoylkojic Acid – Benzoic Acid Addition Compound

(a) Benzoylation in Sodium Hydroxide

A solution of 5 gm. (35 mM.) of kojic acid and 7.3 gm. (177 mM.) of sodium hydroxide was prepared in 177 ml. of water. Benzoyl chloride, 19.7 gm. (140 mM.), was added slowly, with stirring and at room temperature, during two and three-quarter hours, and the granular solid which separated was recovered. A solution of 5.8 gm. (140 mM.) of sodium hydroxide in 50 ml. of water was added to the acidic filtrate, and the benzoylation was repeated with another 140 mM. of benzoyl chloride. After one hour more of stirring, a second crop of the solid was recovered from the liquor, which again had become acid. The two crops when combined and crystallized from dry benzene–hexane yielded 1.55 gm. (18%) of a less soluble fraction which consisted of white crystals with the composition and melting point, 142–143°, of 2-(hydroxymethyl)-5-benzoxy-γ-pyrone (IX). A mixed melting point with an authentic sample was not depressed.

The more soluble fraction was composed of 5.88 gm. (35.5%) of matted white needles melting at 117–118°, raised by further recrystallizations to m.p. 120–121°. Mixtures of this molecular addition compound with dibenzoylkojic acid and with benzoic acid showed depressions in melting point. Found: C, 68.8, 69.0; H, 4.4, 4.4%. Calc. for $C_{20}H_{14}O_6.C_7H_6O_2$: C, 68.6; H, 4.2%.

A solution of 0.50 gm. of the addition compound in 5 ml. of warm acetone was diluted with an equal volume of cold water; the mixture was cooled and the white precipitate which had immediately formed was recovered. The precipitate melted correctly for dibenzoylkojic acid at 134–135°, a mixed melting point with an authentic sample was not depressed, and the yield of 0.37 gm. was 100% of theory. The clear filtrate when concentrated to half-volume deposited 0.12 gm. (92.5%) of benzoic acid melting at 117–119°, raised to the proper value of 122° by recrystallization from water. A mixed melting point was not depressed. Dibenzoylkojic acid also crystallized from solutions of the adduct in dioxane (on dilution with water), 95% ethanol, ethyl acetate, absolute methanol, and pyridine. The benzoic acid was extracted selectively by aqueous 5% sodium carbonate from a solution of the adduct in benzene. On the other hand, the adduct could be recrystallized substantially without change from benzene, benzene–hexane, toluene, and glacial acetic acid.

(*b*) *Benzoylation in Pyridine*

Five grams (36 mM.) of benzoyl chloride was added in several increments to a solution of 2 gm. (14 mM.) of kojic acid in dry pyridine. Next day the pyridine was evaporated in a current of dry air, with gentle heating, to leave a moist pulp. The product in this pulp was separated from the pyridine salts by extraction with hot benzene, and was recovered from the extract in several fractions. The less soluble fractions yielded 3.45 gm. (70%) of pure dibenzoylkojic acid, which was carefully identified as such. Recrystallization of the more soluble fractions from benzene–ligroin gave 0.66 gm. (10%) of white matted needles whose melting point of 120–121° was not depressed by admixture with the dibenzoylkojic acid – benzoic acid addition compound.

The product from a similar benzoylation was isolated in the customary way by pouring the mixture into chilled 5% aqueous sulphuric acid. A 98.5% yield of pure dibenzoylkojic acid was obtained.

2-(Benzoxymethyl)-5-hydroxy-γ-pyrone (*V*)

A solution of 0.30 gm. (0.86 mM.) of dibenzoylkojic acid and 0.12 gm. (1.7 mM.) of hydroxylamine hydrochloride in 4 ml. of pyridine was kept for 12 hr. at room temperature, then diluted with 20 ml. of cold water. After the suspension had been kept cold for a few hours, the white precipitate was recovered. Yield, 0.19 gm. (90%); m.p. 179–180°, increased to 180–181° by recrystallization from ethanol. Found: C, 63.5, 63.6; H, 4.1, 4.2; COC_6H_5, 41.7, 41.0%. Calc. for $C_6H_5O_4(COC_6H_5)$: C, 63.4; H, 4.1; COC_6H_5, 42.7%.

The same product was obtained in 83% yield by boiling dibenzoylkojic acid with three equivalents each of hydroxylamine hydrochloride and pyridine in 95% ethanol for 30 min.; also by replacing the pyridine with sodium acetate and using 80% ethanol. In the latter case the pure product crystallized in 74% yield when the solution cooled. Three equivalents of either sodium acetate or hydroxylamine hydrochloride alone had no appreciable effect on dibenzoylkojic acid in boiling ethanol.

A 15% excess of benzoyl chloride was slowly added to an ice-cold solution of the above monobenzoate in dry pyridine. An 89% yield of dibenzoylkojic acid was recovered, melting at 135°, after crystallization from aqueous acetone. A mixed melting point was undepressed.

2-(Benzoxymethyl)-5-methoxy-γ-pyrone (XII)

One gram (4.1 mM.) of finely powdered 2-(benzoxymethyl)-5-hydroxy-γ-pyrone was added to 18 ml. of benzene containing about 0.5 gm. of diazomethane (2). Practically all of the solid dissolved within six hours, and the product was isolated by concentrating and cooling the solution. The yield was 0.52 gm. (49%) of near-white crystals melting at 109–110°; the use of absorbent charcoal in ethanol, and recrystallization from benzene–ligroin, gave a snow-white product melting at 110–111°. Found: C, 64.7, 64.9; H, 4.6, 4.8; OCH_3, 12.0, 12.1%. Calc. for $C_{13}H_9O_4(OCH_3)$: C, 64.6; H, 4.6; OCH_3, 11.9%.

The same product was obtained when 2-(hydroxymethyl)-5-methoxy-γ-pyrone (VIII), prepared according to Campbell and co-workers (8), was esterified with benzoyl chloride in dry pyridine. A mixture of the two samples showed no depression in melting point.

2-(Acetoxymethyl)-5-benzoxy-γ-pyrone (XIII)

A solution of 2.8 gm. (11 mM.) of 5-benzoxy-2-(hydroxymethyl)-γ-pyrone (IX) in 24 ml. of dry pyridine was chilled, mixed with 4.1 gm. (40 mM.) of acetic anhydride, and kept overnight at room temperature. Precipitation of the solution into 200 ml. of cold water yielded 3.06 gm. (93%) of crystals melting at 143–144°. Recrystallizations from benzene and from aqueous acetone left the melting point of the white plates at 144°, and a mixed melting point with the starting material was depressed to 120–127°. Found: C, 62.8, 62.5; H, 4.3, 4.5%. Calc. for $C_{15}H_{12}O_6$: C, 62.5; H, 4.2%.

A solution of 0.96 gm. (3.3 mM.) of XIII and 0.46 gm. (6.6 mM.) of hydroxylamine hydrochloride in 13 ml. of pyridine was kept at room temperature for 12 hr. before being diluted with six volumes of cold water. After being extracted with benzene, the aqueous liquor was evaporated to dryness *in vacuo* at 50° and the solid, yellow residue was extracted with boiling benzene. The combined benzene extracts yielded a total of 0.47 gm. (77%) of white needles melting at 134–135°, plus a second fraction (6%) of slightly less pure material. 2-(Acetoxymethyl)-5-hydroxy-γ-pyrone (VI), whose published melting point was 133.5° (12) and 137° (17), gave a wine color with aqueous ferric chloride. When acetylated in the presence of sodium acetate (13), the substance yielded 5-acetoxy-2-(acetoxymethyl)-γ-pyrone which was carefully identified by comparison with an authentic sample.

Debenzoylation of 5-Benzoyl-2-(triphenylmethoxymethyl)-γ-pyrone (XI)

The debenzoylation was accomplished as just described, except that an 8.4 molar equivalent of hydroxylamine hydrochloride was used. After recrystallizations from benzene–ligroin, dioxane–water, and carbon tetrachloride – isopropyl ether, 66% of pure 5-hydroxy-2-(triphenylmethoxymethyl)-γ-pyrone (VII) remained, melting at 180–182° (25).

Benzoylallomaltol from Dibenzoylkojic Acid

Thirty grams of technical grade zinc dust was added to a hot solution of 5 gm. (14.3 mM.) of dibenzoylkojic acid in 125 ml. of glacial acetic acid; the mixture was vigorously stirred on the steam bath, and eight drops of concentrated sulphuric acid were added during five minutes. Stirring was stopped after one hour, the hot mixture filtered, the residual zinc rinsed with hot glacial acetic acid, and the combined liquors were evaporated completely *in vacuo*. The residual brown oil was treated with an excess of solid sodium carbonate, the resulting mixture was extracted with boiling benzene, and the benzene extract was washed with dilute aqueous sodium carbonate and with water. Pure benzoic acid, 1.31 gm. (75%), was recovered from the benzene-insoluble solid residue and the sodium carbonate washings. The golden brown benzene solution was completely evaporated and the residual tan solid was recrystallized twice from isopropyl ether, using a small amount of decolorizing charcoal. The yield was 1.48 gm. (45%) of benzoylallomaltol as off-white, flat needles melting at 125–126°. An analytical sample, recrystallized from the same solvent, melted at 126–127°, undepressed when mixed with an authentic sample (24). Found: C, 67.9*, 67.8; H, 4.8*, 4.8%. Calc. for $C_{13}H_{10}O_4$: C, 67.8; H, 4.4%.

A solution of 2.4 gm. (10 mM.) of benzoylallomaltol and 0.95 gm. (14 mM.) of hydroxylamine hydrochloride in 25 ml. of pyridine was kept at room temperature for 11 hr., and was then evaporated *in vacuo*. After being dried over solid potassium hydroxide in a vacuum desiccator, the residual brown oil was extracted several times with boiling benzene. The combined benzene extracts yielded a total of 1.05 gm. of crude allomaltol as white crystals melting at 143–149°; recrystallization from a mixture of isopropyl ether and ethyl acetate left 0.93 gm. (71%) melting at 150–151°. This substance was soluble in water and gave a wine color with ferric chloride. Benzoylation with benzoyl chloride in dry pyridine gave back benzoylallomaltol. An authentic sample of allomaltol prepared by Yabuta's method (24) also melted at 150–151°, not at 166° as reported, and a mixed melting point was undepressed. The present value confirmed that recently given by Looker and Okamoto (16).

Dibenzoylkojic Acid "Disemicarbazone"

(*a*) A mixture of 14 gm. (40 mM.) of dibenzoylkojic acid, 14 gm. (125 mM.) of semicarbazide hydrochloride, 14 ml. (174 mM.) of pyridine, 40 ml. of water, and 350 ml. of ethanol was boiled under reflux for 50 min. and then kept at room temperature for six days. The crop of yellow-white crystals deposited from the clear solution was extracted in succession with hot ethyl acetate, water, and dioxane, and the residual microcrystalline white solid, 6.04 gm., melted at 212° with decomposition. A second crop, recovered from the original mother liquor 15 days later, increased the yield to 7.96 gm. or to 41.5%. This "disemicarbazone" was sparingly soluble in glacial acetic acid but insoluble in all other liquids tried. The sample for analysis was dissolved in much glacial acetic acid by warming to not more than 70° and was reprecipitated by adding five volumes of cold water. The melting point was then 215° with decomposition. Found: C, 54.8, 54.8; H, 4.7, 4.8; N, 16.9%. Calc. for $C_{22}H_{22}N_6O_7$: C, 54.8; H, 4.6; N, 17.4%.

(*b*) The above condensation was repeated with 5 gm. of dibenzoylkojic acid, 3.2 gm. of semicarbazide hydrochloride, 5 ml. of pyridine, 10 ml. of water, and

60 ml. of ethanol, the molar ratio of the first three substances being about 1:2:4 instead of about 1:3:4. The yellow solution deposited a yellow-white solid decomposing at 179–181° when concentrated on the steam-bath, and a soft solid separated when the mother liquor was diluted with water and chilled. Extraction of the latter fraction with boiling ethyl acetate removed 0.33 gm. of a solid, m.p. 183–184°, which was combined with the previous fraction of similar melting point. These fractions were purified by extraction with hot ethyl acetate and water to yield 1.65 gm. (24%) of the "disemicarbazone" noted in (*a*) and melting at 213°.

On cooling overnight, the red ethyl acetate extract deposited 0.61 gm. of white crystals melting at 121° with decomposition. Recrystallizations from ethyl acetate and from ethanol–methanol left 0.4 gm. (6%) of this isomeric "disemicarbazone" and raised the melting point to 172–172.5° with decomposition. Found: C, 54.6, 54.5; H, 5.0, 4.9; N, 16.6%. Calc. for $C_{22}H_{22}N_6O_7$: C, 54.8; H, 4.6; N, 17.4%.

Experiments with the Higher Melting "Disemicarbazone"

(*a*) *Action of Dilute Alkali*

Two grams of the above "disemicarbazone" dissolved almost completely when stirred at room temperature for 30 min. with 20 ml. of 10% potassium hydroxide. The clear filtrate contained no substances that could be extracted by ether or chloroform. When acidified with hydrochloric acid, the filtrate deposited 0.95 gm. (95%) of benzoic acid with the correct melting point and mixed melting point.

(*b*) *Action of Dilute Acid*

A suspension of 1.5 gm. of the "disemicarbazone" in 25 ml. of 10% hydrochloric acid was boiled under reflux for three and one-half hours. The sticky brown residue was separated from the acidic filtrate (which yielded 0.31 gm., or 41%, of benzoic acid) and was crystallized from methanol–ethanol–benzene, from dioxane–water, and from acetone. The pure product, 0.11 gm. or 9%, was a white, microcrystalline solid melting at 244°. Found: C, 64.3, 64.5; H, 4.1, 4.2; N, 10.8%; mol. wt. (Rast), 423. Calc. for $C_{21}H_{15}N_3O_5$: C, 64.8; H, 3.9; N, 10.8%; mol. wt. 389.

(*c*) *Oxidation with Potassium Permanganate*

A mixture of 5.61 gm. (11.6 mM.) of the "disemicarbazone", 10 gm. (63 mM.) of potassium permanganate, 4 ml. (70 mM.) of glacial acetic acid, and 370 ml. of water was kept at 90° for 30 min. After removing the manganese dioxide, the weakly acid filtrate was concentrated to 250 ml. *in vacuo*, was adjusted to pH 2, and was continuously extracted with benzene. The residue from the extract, when fractionally crystallized from benzene–hexane, yielded 0.38 gm. (27%) of pure benzoic acid, and 0.24 gm. (11.5%) of benzoylglycolic acid melting correctly (7) at 111–112°. Found for the latter: C, 60.0; H, 4.8%*; neut. equiv., 179.7. Calc. for $C_9H_8O_4$: C, 60.0; H, 4.4%; neut. equiv., 180.

Dibenzoylkojic Acid "α-Monosemicarbazone"

A suspension of 3.05 gm. (6.33 mM.) of the "disemicarbazone" in 70 ml.

of glacial acetic acid was stirred at room temperature, while a concentrated aqueous solution of 10.8 gm. (157 mM.) of sodium nitrite was added very slowly during six hours. Stirring was discontinued one hour later, and next day the yellow-green solution was filtered from 0.3 gm. of the starting material. Dilution of the filtrate with an equal volume of cold water precipitated 2.03 gm. of a white solid melting at 173–174°. This solid contained several components, but only one could be isolated in a pure form by recrystallizing the mixture from benzene–methanol (1 vol. : 4 vol.). The product, 0.24 gm. (9%), was a white microcrystalline powder melting with decomposition at 212–213°, increased to 215° by recrystallizations from acetone–methanol. A mixed melting point determination with the original "disemicarbazone" (m.p. also 215°) was markedly depressed. Found: C, 59.6, 59.0; H, 4.7, 4.6; N, 10.1%. Calc. for $C_{21}H_{19}N_3O_7$: C, 59.3; H, 4.5; N, 9.9%.

Dibenzoylkojic Acid "β-Monosemicarbazone"

The preparation for the α-isomer was repeated with 7.2 gm. (104 mM.) of sodium nitrite instead of 10.8 gm., and with 60 ml. instead of 70 ml. of glacial acetic acid. The filtered liquor on dilution with 240 ml. of cold water deposited 2.41 gm. of a white solid decomposing at 166–170°. This product was extracted with 120 ml. of boiling methanol to remove a small amount of unchanged "disemicarbazone". The filtered extract, when diluted with 500 ml. of water, deposited 1.82 gm. (69%) of a white, microcrystalline solid whose decomposition point at 178–179° was not altered by recrystallization from benzene–methanol–ligroin. Found: C, 59.3, 59.2; H, 5.1, 4.9; N, 9.8%. Calc. for $C_{21}H_{19}N_3O_7$: C, 59.3; H, 4.5; N, 9.9%. A sample was recovered unchanged after its suspension in glacial acetic acid had been treated with an excess of aqueous sodium nitrite at room temperature.

Another sample, 0.60 gm. (1.4 mM.), was heated on the steam bath with 1.1 gm. (7 mM.) of potassium permanganate and 40 ml. of water for one and three-quarter hours. The reaction mixture, when worked up as already described for the oxidation of the "disemicarbazone", yielded 0.05 gm. (19.5%) of benzoylglycolic acid melting at 106–110°. Recrystallization raised the melting point to 111–112°, undepressed by admixture with the previous sample. The other product of the oxidation was benzoic acid, which was recrystallized until pure.

ACKNOWLEDGMENTS

One of us (A. B.) wishes to thank the National Research Council of Canada for a Studentship, and the Pulp and Paper Research Institute of Canada for a summer stipend. We acknowledge gratefully the determination of the infrared spectra of the dibenzoylkojic acid – benzoic acid adduct by Mr. A. W. Pross of Canadian Industries (1954) Limited, McMasterville, Que.; also a generous gift of kojic acid from the Corn Products Refining Company, Argo, Ill.

REFERENCES

1. AINSWORTH, C. and JONES, R. G. J. Am. Chem. Soc. 76: 3172. 1954.
2. ARNDT, F. Organic syntheses. Vol. XV. John Wiley & Sons, Inc., New York. 1935. p. 4, Note 7.

3. ARNSTEIN, H. R. V. and BENTLEY, R. J. Chem. Soc. 3436. 1951.
4. BARHAM, H. N. and SMITS, B. L. Trans. Kansas Acad. Sci. 37: 91. 1934.
5. BEDEKAR, D. N., KAUSHAL, R. P., and DESHAPANDE, S. S. J. Indian Chem. Soc. 12: 465. 1935.
6. BEÉLIK, A. *In* Advances in carbohydrate chemistry. Vol. 11. *Edited by* M. L. Wolfrom. John Wiley & Sons, Inc., New York. To be published in 1956.
7. BRIGL, P. and GRÜNER, H. Ber. 65: 641. 1932.
8. CAMPBELL, K. N., ACKERMAN, J. F., and CAMPBELL, B. K. J. Org. Chem. 15: 221. 1950.
9. GOLDSCHMIDT, S. and VEER, W. L. C. Rec. trav. chim. 65: 796. 1946.
10. HAITINGER, L. and LIEBEN, A. Monatsh. 5: 339. 1884.
11. HEYNS, K. and VOGELSANG, G. Ber. 87: 1377. 1954.
12. HEYNS, K. and VOGELSANG, G. Ber. 87: 1440. 1954.
13. HURD, C. D. and SIMS, R. J. J. Am. Chem. Soc. 71: 2440. 1949.
14. INGOLD, C. K. Structure and mechanism in organic chemistry. Cornell Univ. Press, Ithaca, N.Y. 1953. pp. 206, 214.
15. INGOLD, C. K. and SHOPPEE, C. W. J. Chem. Soc. 1868. 1928.
16. LOOKER, J. H. and OKAMOTO, T. T. Abstr. of Papers, Am. Chem. Soc. 125: 9N. 1954.
17. MAURER, K. Ber. 63: 25. 1930.
18. PETUELY, F. and KÜNSSBERG, U. Monatsh. 83: 80. 1952.
19. SCHÖNBERG, A. and SINA, A. J. Am. Chem. Soc. 72: 1611. 1950.
20. WILLSTÄTTER, R. and PUMMERER, R. Ber. 38: 1461. 1905.
21. WOLFROM, M. L. Rec. trav. chim. 66: 238. 1947.
22. YABUTA, T. J. Chem. Soc. Japan, 37: 1185. 1916. Chem. Abstr. 17: 1475. 1923.
23. YABUTA, T. J. Chem. Soc. Japan, 37: 1234. 1916. Chem. Abstr. 17: 1475. 1923.
24. YABUTA, T. J. Chem. Soc. 125: 575. 1924.
25. YABUTA, T. and ANNO, K. J. Agr. Chem. Soc. Japan, 23: 104. 1949. Chem. Abstr. 44: 3492. 1950.
26. YALE, H. L. Chem. Revs. 33: 209. 1943.