SOME CARBOXYLIC ACIDS PRESENT IN ROYAL JELLY[1]

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ABSTRACT

Only one carboxylic acid, 10-hydroxy-2-decenoic acid, has previously been isolated from royal jelly. Small amounts of two additional acids, sebacic and 2-decendioic acid, have now been found in the ether-soluble portion of this special bee foodstuff. Evidence is presented in favor of the *trans*-configuration for naturally occurring 10-hydroxy-2-decenoic acid. Chromic acid oxidation of the hydroxy acid yields 2-decendioic acid.

The chemical nature of the components of royal jelly has received considerable attention over the past 20 years. Townsend and Lucas (1) reported the isolation of a carboxylic acid, $C_{10}H_{18}O_3$, from the mixture of acids obtained from royal jelly by extraction with ether. Abbott and French (2) and Abbott (3) confirmed this finding and attempted by oxidative means to establish a structure for the acid. However, their efforts were unsuccessful. More recently Butenandt and Rembold (4) have characterized the compound as 10-hydroxy-2-decenoic acid (I).

This year Barker, Foster, and Lamb (5) have independently confirmed the structure proposed by Butenandt and Rembold for I and have speculated on the olefinic configuration.

In view of the increasing interest in royal jelly from a therapeutic standpoint and the findings recently reported by Townsend, Morgan, and Hazlett (6) on the activity of I against experimental leukaemia and ascitic tumors we are reporting the results of our investigation into the chemical nature of additional carboxylic acids which we have discovered in royal jelly.

Besides 10-hydroxy-2-decenoic acid (I) we have isolated, by chromatographic means, sebacic acid (II) and 2-decendioic acid (III) from the mixture of acids obtained from lyophilized royal jelly by ether extraction.

$$HO—CH_2—(CH_2)_6—CH=CH—COOH \qquad I$$
$$HOOC—(CH_2)_6—CH_2—CH_2—COOH \qquad II$$
$$HOOC—(CH_2)_6—CH=CH—COOH \qquad III$$

Barker *et al.* (5) have reported that I comprises approximately 70% of the mixture of ether-soluble acids and approximately 15% of royal jelly. We find that I is present to a greater extent in the mixture of ether-soluble acids, 90%, whereas it is present only in the amount of 3% in whole royal jelly (7% of lyophilized royal jelly). A variety of melting points have been reported for this compound: 46–58° (1); 61–62° (3); 54–56° (4); 52° (5). When I is separated chromatographically from the other ether-soluble acids with which it is associated in royal jelly and subsequently crystallized from water, the pure material has m.p. 62.0–62.5°.

Only Barker *et al.* (5) have considered the olefinic configurations of I but were unable to make a firm assignment. We have found that I, when irradiated in acetone solution, is converted slowly to another isomer (IV). This latter compound, m.p. 43–46°, slowly reverts to I when allowed to stand at room temperature even in the solid state. In the case of IV, absorption is strong in the region 300–340 mμ while I is relatively transparent

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over the same range. Both I and IV show strong end absorption (220–290 mμ) in the ultraviolet. Infrared absorption spectra of I and IV were run on mineral oil mulls and are summarized in Table I.

TABLE I

Infrared spectra of 10-hydroxy-2-decenoic acid

Peaks, cm^{-1}	
Naturally occurring (I)	Irradiated with sunlight (IV)
3390	3390
1710	1710
1658	1658
976	976
—	830

The medium peak observed at 830 cm^{-1} in the spectrum of IV is not present in that of I. Allan, Meakins, and Whiting (7) have determined the infrared absorption spectra of a number of α,β-unsaturated carboxylic acids and have established that a peak in the region 830 cm^{-1} indicates an olefinic C—H out-of-plane bending for *cis*-α,β-unsaturated acids. The presence of a peak at 976 cm^{-1} in the spectra of both I and IV indicates that IV is contaminated with I (incomplete conversion). A peak in this latter region has been well established as being characteristic of *trans*-substituted ethylenic structures (8). Based on these observations we postulate that naturally occurring 10-hydroxy-2-decenoic acid exists in the *trans*-form.

The presence of sebacic acid (II) in royal jelly is interesting since its occurrence in other natural foodstuffs is limited to the fat portion of milk from buffalo and cow (9). It has been reported by Stählin and Blievernicht (10) that the free sebacic acid content of rape and turnip seed rises as it becomes moldy.

The third acid, 2-decendioic acid (III), found in royal jelly, has previously been reported only twice. It was synthesized by English (11), who was investigating homologues of traumatic acid ($HOOC-(CH_2)_8-CH=CH-COOH$). The latter compound is a factor involved in plant growth phenomena.

Cram and Tishler (12) isolated III from the acetone-soluble fraction of clinical penicillin. Chromic acid oxidation of 10-hydroxy-2-decenoic acid (I) readily yields III. This reaction was originally reported by Abbott (3), but no structures were known at that time.

It is significant that the three acids in royal jelly are all closely related, 10-carbon compounds. The concentration of each acid is given in Table II.

TABLE II

Acid	Conc. (p.p.m.) (basis lyophilized royal jelly)
I	70,000
II	200
III	300

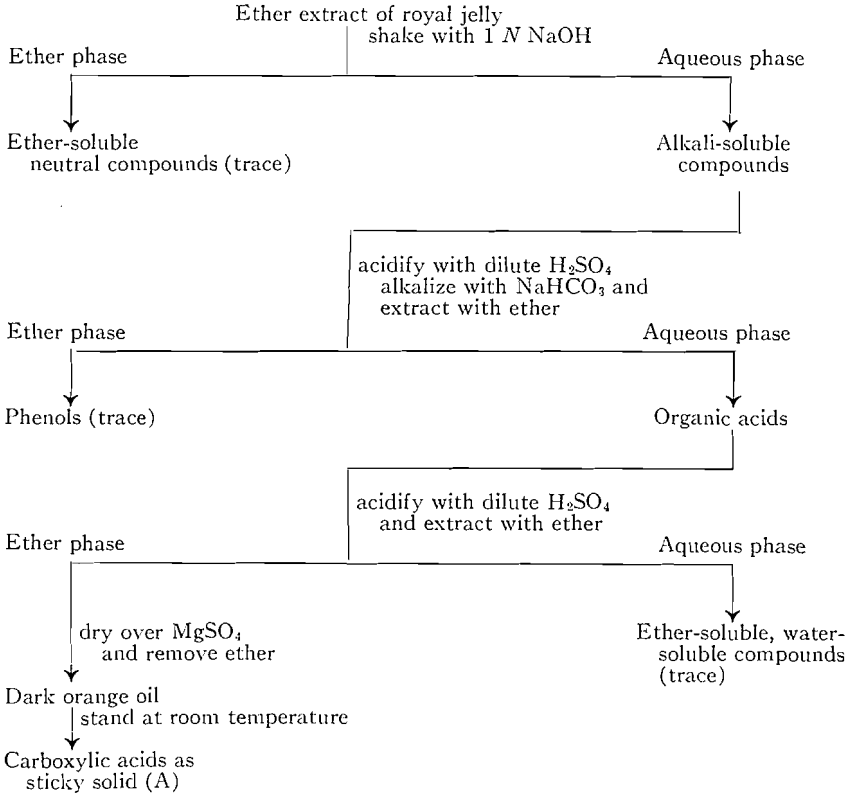
The chemotherapeutic value of II and III is being investigated by our co-workers, Townsend, Morgan, and Hazlett (6) and will be reported elsewhere.

EXPERIMENTAL

(All melting points have been corrected against reliable standards.)

Isolation of Carboxylic Acids (A) from Royal Jelly

Lyophilized royal jelly was placed in a Pyrex 3885 Soxhlet extractor in 2-inch layers alternated with glass wool and was extracted with ether for 26 hours. The resulting straw-colored extract, approximately 2 l. in volume, was concentrated to 200 ml and processed as shown in the following flowsheet.



The final ether extract was dried over anhydrous magnesium sulphate, filtered, and the ether removed in a current of air. The residue, (A), a dark orange oil, crystallized after standing overnight at 25°.

A number of samples of lyophilized royal jelly extracted with ether and the ether extract processed as already described showed an average of 7% ether-soluble organic acids. This represents 3% of whole royal jelly which averages 65% moisture and 35% solids.

Isolation and Identification of Sebacic Acid (II) *as the p-Bromophenacyl Ester*

The crystalline mixture of acids, (A), (7.7 g), was taken up in hot hexane–ether and refrigerated to 5°. While the solution was cooling, seeds of 10-hydroxy-2-decenoic acid were introduced. The resulting white crystalline solid, 3.8 g, was removed by filtration, m.p. 43–46°. A further crystallization of the solid gave 2.8 g (36% by wt.) of impure 10-hydroxy-2-decenoic acid, m.p. 55–59°. The mother liquor from the first crystallization

was concentrated and cooled, yielding 1.7 g of white crystalline solid, m.p. 39–63°. A second crystallization of the latter solid using the same mixed solvent gave 1.2 g of white crystals, m.p. 45–72°. Crystallization from benzene–hexane raised the m.p. to 51–78°.

When 0.37 g of the material, m.p. 51–78°, was treated with *p*-bromophenacyl bromide by the usual procedure, a white solid was formed in the hot reaction mixture and was removed by filtration before cooling. It weighed 0.050 g, m.p. 146–150°. Crystallization from absolute ethanol and acetone raised the m.p. to 150–151°.

A mixture melting point of the latter compound with authentic *p*-bromophenacyl ester of sebacic acid was not depressed.

Isolation and Identification of Sebacic Acid (II) *and 2-Decendioic Acid* (III) *from A by Chromatography*

Isolation of III was achieved by chromatographic separation of A on Whatman seed test paper (1/16 inch) using a 7:3 ratio by volume of propanol and concentrated aqueous ammonia as the developing solvent. Isherwood and Hanes (13) have described the use of this solvent system.

A concentration of 700 mg of A per 9×22.5-inch sheet gave adequate separation of II and III from I. Acid I comprises approximately 90% of A. The various regions containing the acids were located on the paper by cutting out a quarter-inch strip from the center of the sheet and spraying it with a 0.1% solution of thymol blue adjusted to pH 10. No significant separation of II from III was achieved at this concentration.

The region containing acids II and III was cut out and the acids extracted from the paper with propanol in a Soxhlet apparatus. The extract was evaporated to dryness, the residue taken up in 5% aqueous sodium hydroxide solution, acidified with dilute sulphuric acid, and extracted with ether. From 2.7 g of A, 91 mg of the mixture of II and III was obtained. Fractional crystallization from chloroform removed most of II. The resulting crude III was fractionated on Whatman 3 MM chromatography paper using the same solvent system as previously described to remove the residual II.

Compound III was extracted from the paper, m.p. 161–168°. After crystallization from chloroform 25 mg of pure III was obtained, m.p. 172–173°.

The infrared spectrum, mull in mineral oil, of III shows maximum absorptions at 1,710 cm^{-1} (carboxylic acid), 1,655 cm^{-1} (unsaturation), and 1,198 cm^{-1} (carboxylic acid). Anal. calc. for $C_{10}H_{16}O_4$: C, 60.0; H, 8.00. Found: C, 59.9; H, 7.92.

The identity of III was confirmed by mixture melting point with 2-decendioic acid, m.p. 172–173°, prepared by chromic acid oxidation of 10-hydroxy-2-decenoic acid.

Sebacic acid (II) was also isolated by the same procedure and identified as the *p*-bromophenacyl ester.

Using Whatman No. 1 chromatography paper and the same developing solvent as before, R_f values are I, 0.79; II, 0.43; and III, 0.38 at 25°.

Oxidation of 10-Hydroxy-2-decenoic Acid (I) *to 2-Decendioic Acid* (III)

A mixture of 0.93 g (0.0050 mole) of 10-hydroxy-2-decenoic acid and 1.0 g (0.0034 mole) of potassium dichromate, dissolved in a solution of 0.9 ml of C.P. concentrated sulphuric acid in 15 ml of water, was heated in a 50-ml round-bottomed flask on a steam bath for 3 hours. After the reaction mixture was allowed to stand at room temperature for 10 hours a solid product was removed by filtration. It weighed 0.43 g, m.p. 145–160°. The crude material was crystallized from hot water including treatment with activated charcoal. In this manner 0.20 g (20%) of small white rosettes, m.p. 171–172° was obtained.

Effect of Ultraviolet Irradiation on Naturally Occurring 10-Hydroxy-2-decenoic Acid (I)

When 10-hydroxy-2-decenoic acid (I), m.p. 62°, isolated from A, was irradiated with sunlight in a thin-walled Pyrex bulb in acetone solution for a total of 20 hours, the melting point of I dropped to 43–46°, suggesting a conversion of the acid from *trans*- to *cis*-configuration. After the solution had been standing for a three-week period the melting point of the irradiated material (IV) rose to 44–54° indicating slow reversion to the more stable *trans*-isomer.

p-Bromophenacyl Ester of 10-Hydroxy-2-decenoic Acid (I)

The *p*-bromophenacyl ester of I has been prepared by Abbott (3) but no melting point has been reported. The ester is readily obtained by treating the sodium salt of I with *p*-bromophenacyl bromide in hot dilute ethanol solution. It has m.p. 89–90°.

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