The Chemical Structure of a Component of *Citrullus colocynthis*, SCHRAD.

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A new substance, molecular formula $C_8H_{10}O_2$, was isolated from the unripe fruits of *Citrullus colocynthis*, SCHRAD. Judging from the results of infrared absorption spectra, properties of the derivatives and the oxidative product of methyl derivative, this substance was presumed to be *p*-hydroxybenzyl methyl ether and this assumption was proved beyond doubt by its direct comparison with an authentic synthesized sample.

Citrullus colocynthis, SCHRAD is a kind of wild perennial liana indigenous to Southeast Asia and Africa. The extract of its fruit is called colocynth, which has long been used as a purgative or a vermifuge.

In recent years Lavie et al.¹⁾ studied the chloroform extract of this fruit and made known that the extract had an anti-cancer property. The active substance is a glucoside, which yielded an aglucone $C_{30}H_{44}O_7$ on hydrolysis. Recently²⁾ the latter substance was found to be identical with the one obtained from *Ecballium elaterium*, A. RICH. On the other hand one of the present authors (Ogawa) and his collaborators made genetic and biochemical studies^{3,4)} on a bitter constituent of *Citrullus colocynthis*, SCHRAD., which they named "citbittol", and found that this substance is composed of eight components⁵⁾ and has anti-cancer properties⁶⁾.

Lately the present authors have made a thorough chemical investigation of the components of *Citrullus colocynthis*, SCHRAD., and succeeded to isolate crystalline plates of m.p. 85~6°C, beside a glucoside, which is probably the same as the one described by Lavie. The chemical structure of this crystalline substance, which was hitherto unknown in the literature, was now elucidated and the details are given below.

For isolation of this compound, unripe fruits, about 7 or 8 days after bearing, were collected and worked up as follows; Fig. 1.

From the molecular weight and analytical data, a molecular formula $C_8H_{10}O_2$ was attributed to this substance.

As can be understood from the method of isolation given above, this substance is phenolic and moreover as such it gives positive test with ferric chloride and also with diazotized benzenesulfanilic acid and sodium carbonate.

From the infrared data the presence of phenolic OH was also revealed (3.08, 7.40, 7.85 μ) and the bands at 9.37 μ , 12.03 μ and 7.22 μ may be associated with an ether linkage, a 1,4-disubstituted benzene ring and a methyl group respectively. The phenolic nature was

1) D. Lavie, D. Willner, M. Belkin and W.G. Hardy, Abstract of symposium on the chemotherapy of cancer, (Tokyo, October, 1957).

2) D. Lavie and D. Willner, *J. Am. Chem. Soc.*, **82**, 1668 (1960).

3) Y. Ogawa, and M. Shimotsuma, *Ann. Rep. Nat. Inst. Gen.*, **9**, 93 (1958).

4) M. Shimotsuma and Y. Ogawa, *Jap. J. of Gen.*, **35**, (5, 6), 143 (1960).

5) Y. Ogawa, and K. Furusato, *Ann. Rep. Nat. Inst. Gen.*, **9**, 92 (1958).

6) Y. Ogawa, *Ann. Rep. Nat. Inst. Gen.*, **9**, 94 (1958).

FIG. 1.

also supported from the fact that this substance gave mono *p*-toluenesulfonate and mono *p*-nitrobenzoate, both of which gave satisfactory analyses as such and are devoid of OH infrared absorption band.

The corresponding methyl ether, prepared with diazomethane, furnished *p*-methoxybenzoic acid by oxidation with potassium per-

manganate.

Judging from the above mentioned data this substance was presumed to be *p*-hydroxybenzyl methyl ether and this assumption was proved by direct its comparison with an authentic specimen synthesized as follows; Fig. 2.

The ultimate compound thus prepared melted at 85~6°C alone or admixed with the natural product. Their infrared spectra also were identical.

EXPERIMENTAL

Extraction and Isolation of (D)

Thirteen liters of methanol were added to the 19.4 kg of crashed unripe fruits of *Citrullus colocynthis*, SCHRAD. After standing at room temperature for twenty four hrs. the mixture was filtered by press. The residue was again extracted with 13 l of methanol and filtered. The combined filtrate was concentrated under reduced pressure to 4 l and extracted with 4 l of chloroform three times. The chloroform layer was concentrated to dryness under reduced pressure and treated with 200 ml of ether to remove 10.1 g (0.052% of raw material) of insoluble substance. The insoluble substance was crude glucoside. The ethereal layer was extracted with dil. sodium bicarbonate solution to remove acids, and then extracted with 5 per cent sodium hydroxide solution to extract phenolic substances. The alkaline layer was acidified with dil. sulfuric acid and extracted with ether. The extract was distilled under reduced pressure to yield 0.25 g (0.0015%) of (D); b.p. 95~100°C. The distillate, soon solidified, was recrystallized from benzene to give a colorless plate; m.p. 85~86°C. M.W. Found: 138. 141. 139. (by K. Rast method) Calcd. for $C_8H_{10}O_2$: 138. Anal. Found: C, 69.99; H, 7.25. Calcd. for $C_8H_{10}O_2$: C, 69.54; H, 7.31%.

FIG. 2.

FIG. 3. Infrared Absorption Spectra of Natural and Synthesized *p*-Hydroxybenzyl Methyl Ether.

———— Natural, - - - - - Synthesized

p-Toluenesulfonate

To a solution of 20 mg of (D) in 0.2 ml of absolute pyridine, 30 mg of *p*-toluenesulfonylchloride in 0.2 ml of absolute pyridine was added under cooling. After standing for twelve hrs. at room temperature 5 ml of ice water was added. The separated oil immediately solidified. The crystal was filtered and recrystallized from 70 per cent ethanol to give a colorless needle; m.p. 45~46°C. *Anal.* Found: C, 61.59; H, 5.52. Calcd. for $C_{15}H_{10}O_4S$; C, 61.64; H, 5.52%.

p-Nitrobenzoate of (D)

This experiment was carried out in the same procedure as above described. *p*-Nitrobenzoate was obtained as a slight yellow crystal: m.p. 92~93°C. *Anal.* Found: C, 63.56; H, 4.22; N, 4.86. Calcd. for $C_{15}H_{13}O_5N$: C, 62.71; H, 4.56; N, 4.88%.

Oxidation of the Methyl Ether Derivative of (D)

Eighty seven mg of (D) was added to an excess of ethereal diazomethane solution and allowed to stand for twenty four hrs. at room temperature. After evaporating the solvent and the excess of diazomethane, the residue was dissolved in 10 ml of ether, shaken with 2 per cent sodium hydroxide solution to remove the original substance, washed with water, dried, evaporated and distilled under reduced pressure to yield 58 mg of colorless liquid: b.p.₆ 90~95°C (oil bath temperature). Forty mg of the methyl ether derivative in 4 ml of 50 per cent aqueous pyridine was oxidized to carboxylic acid with 160 mg of potassium permanganate in 10 ml of water dropping in a period of twenty minutes. The solution was heated on water bath until the color of permanganate disappeared. After the oxidation of the methyl ether derivative was completed, the separated manganese dioxide was filtered by suction and washed with acetone. The combined filtrate was concentrated under reduced pres-

sure to 3 ml and acidified with hydrochloric acid to conformed reaction. The separated crystals were filtered and recrystallized from ethanol to give a needle crystal: m.p. 184°C. The mixed melting point with the authentic *p*-methoxybenzoic acid was not depressed.

Synthesis of *p*-Hydroxybenzyl Methyl Ether.

Two point seven gr of *p*-aminobenzyl methyl ether were dissolved in 7.4 g of 10 per cent hydrochloric acid and diluted to 100 ml with water. To this solution, 3.5 g of sodium nitrite in 15 ml of water was added at room temperature. The mixture was stirred for one hr. at room temperature, for thirty minutes at 70°C, and finally for thirty minutes at 90~95°C. After cooling the separated oily substance was extracted with ether and the ethereal layer was extracted with 5 per cent sodium hydroxide solution. The alkaline solution was acidified with hydrochloric acid and extracted with ether. The ethereal layer was dried, evaporated and distilled under reduced pressure to yield 0.4 g; b.p.₁ 95~100°C. The distillate, soon solidified, was recrystallized from benzene to give a colorless plate: m.p. 85~86°C. The mixed melting point with the natural substance was not depressed. *Anal.* Found: C, 69.68; H, 6.95. Calcd. for $C_8H_{10}O_2$: C, 69.54; H, 7.30%.

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