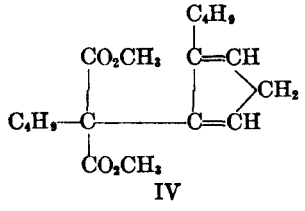
allene absorption at 1920 cm.$^{-1}$ and the terminal methylene group absorption bands near 1635 and 900 cm.$^{-1}$, were absent. Two new, strong and sharp absorption bands at 1620 and 1670 cm.$^{-1}$ were found which we attribute to the presence of conjugated double bonds of a cyclopentadienyl ring in IV.



IV

The presence of the cyclopentadienyl ring is also favored by the fact that IV reacted with maleic anhydride and that a highly red-colored product (substituted fulvene) was obtained when IV was condensed with benzaldehyde.

The half esterification of IIA yielded an acid which contained all the characteristic bands of IIA. The carbonyl group absorption region showed two absorption bands at 1725 and 1680 cm.$^{-1}$, due to the ester and acid linkages.

The esterification of III also yielded two moles of nitrogen and the product contained a terminal methylene group as evidenced by the bands at 1625 and 910 cm.$^{-1}$ The half esterification of III produced a monobasic acid with two different carbonyl groups (bands at 1725 and 1690 cm.$^{-1}$) and a terminal methylene group (1630 and 912 cm.$^{-1}$).

Hydrogenation studies have shown the presence of three double bonds, esterification with diazomethane the presence of two carboxy groups, and the titration studies are in accord with the behavior of other highly branched malonic acids.[5] On that basis we are assigning the dimeric acid to have the structure IIA, a structure previously considered[4] but not adopted.

EXPERIMENTAL

IIA. The preparation was previously described,[4] m.p. 99–100°: The esterification was carried out by treating 0.7043 g. (0.00251 mole) of IIA with 0.00595 mole of diazomethane in ether. The volume of evolved nitrogen was 119 ml. (S.T.P.), 105% of theory based on two acid groups. The product distilled at 178° at 17 mm.

Anal. Calcd. for $C_{18}H_{28}O_4$: C, 70.1; H, 9.1. Found: C, 70.1; H, 9.1.

The distillate gave a deep red colored solution when treated with benzaldehyde in the presence of sodium ethoxide. When refluxed with a saturated solution of maleic anhydride in toluene, a white crystalline solid separated.

The half esterification was carried out by treating 0.3769 g. (0.001345 mole) of IIA with 0.00159 mole of diazomethane dissolved in ether. The evolved nitrogen, 31.5 ml. (S.T.P.), was 52% of theory based on two acid groups. The ether was evaporated and the crude residue had a neutralization equivalent of 304.

Anal. Calcd. for $C_{17}H_{26}O_4$: neut. equiv. 294.

III. The preparation was previously described.[4] The dimethyl ester was prepared by treating 0.6672 g. (0.00235 mole) of III with an excess of diazomethane. The volume of nitrogen was 102 ml. (S.T.P.) which is 97% of theory based on two acid groups. Upon the evaporation of the ether, the product boiled at 132–134° at 4 mm.

Anal. Calcd. for $C_{18}H_{32}O_4$: C, 69.2; H, 10.2. Found: C, 70.6; H, 9.7.

The monomethyl ester was prepared as previously described.[4]

Anal. Calcd. for $C_{17}H_{30}O_4$: neut. equiv. 298. Found: neut. equiv. 296.

In the complete hydrogenation of IIA, 0.0812 g. (0.00029 mole) of IIA absorbed 19.7 ml. (S.T.P.) of hydrogen in 100 min. when an "active" lot of platinum oxide catalyst (0.0077 g.) was used. After the removal of the acetic acid solvent, the residue was crystallized from petroleum ether, m.p. 104–105°.

Anal. Calcd. for $C_{16}H_{30}O_4$: C, 67.1; H, 10.5; mol. wt. 286. Found: C, 66.0; H, 10.1; mol. wt. 304 (titration), 277 (cryoscopic).

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Ammajin, a New Constituent of *Ammi Majus* (L.)

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The fruits of the umbelliferous plant *Ammi majus* (L.) which grows freely in the Nile Delta have been used for centuries as a remedy for leukoderma (vitiligo). The active constituents responsible for the photodynamic properties of this Egyptian plant have been identified with the furocoumarins xanthotoxin, bergapten and imperatorin.[1] Recently, there was an increase of interest in the physiologically active furocoumarins because of the development of the psoralen (furocoumarin) treatment of pigmentation diseases[2a] and alopecia areata,[2b] and the discovery of the effect of these photosensitizing agents on skin carcinogenesis.[2c]

As many plants containing free furocoumarins have, among their other constituents, glycosidic compounds whose aglucones are related coumarins, it was worth studying the glycoside fraction of the fruits of *Ammi majus* (L.) and isolating such compounds. It has been reported in the literature that the fruits of this plant contain about 1% of an amorphous glucosidal principle,[3] but until now no

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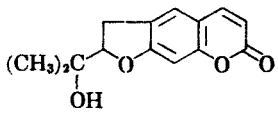
(1) I. R. Fahmy, H. Abu-Shady, A. Schönberg, and A. Sina, *Nature*, **160**, 468 (1947); I. R. Fahmy and H. Abu-Shady, *Quart. J. Pharm. and Pharmacol.* **20**, 281 (1947); *Quart. J. Pharm. and Pharmol.* **21**, 499 (1948).

(2) (a) Comp. E. Sidi, J. Bourgeois-Spinasse, and P. Planat, *Dyschromies et Vitiligo*, Expansion scientifique française, Paris, France, 1957; (b) E. Sidi and J. Bourgeois-Spinasse, *Presse med.*, **63**, 458 (1955); (c) M. A. O'Neal and A. C. Griffin, *Cancer Research* **17**, 911 (1957).

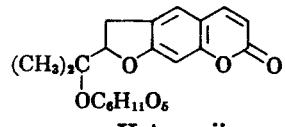
(3) I. R. Fahmy and M. A. El Keiy, *Rep. Pharm. Soc. Egypt*, **3**, 72 (1931).

crystalline substance has been isolated from this material. The study of the aqueous fraction obtained by concentrating the aqueous alcoholic extract of the seeds of *Ammi majus* (L.) has revealed the presence of a crystalline glycoside $C_{20}H_{24}O_9$, melting at 260°, having an optical rotation $[\alpha]_D^{25}$ −60° and showing the characteristic properties of coumarins. By acid hydrolysis, this compound gave D-glucose and an optically active aglucone $C_{14}H_{14}O_4$ (I), identical with the coumarin marmesin occurring in the bark of the tree *Aegle marmelos* Correâ.[4] Marmesin is the optical antipode of nodakenetin, which occurs in nature as a glucoside, nodakenin[5] ($[\alpha]_D^{30}$ +56.6°[5]; $[\alpha]_D^{18}$ +57°[6]). However, the isolation of a glycoside of marmesin has not been reported up to the present time.

The proposed structure for the new glucoside, which we have named ammajin (II), is supported by the partial synthesis of this product from its aglucone marmesin and D-glucose, by the method based on the reaction of hydroxy compounds with pentaacetylglucose under the catalytic action of toluenesulfonic acid.[6]



I Marmesin



II Ammajin

EXPERIMENTAL[7]

Extraction of ammajin (II) *from the seeds of Ammi Majus* (L.). Two hundred grams of the finely powdered seeds were exhausted by percolation with 70% ethanol and the extract distilled in vacuum to remove the alcohol. After extraction of the remaining residue with chloroform in order to remove the last traces of the substances soluble in this solvent, the sirupy liquid thus obtained (50 ml.) was treated with a mixture of lead acetate and lead hydroxide (prepared by mixing 5 g. of lead acetate with 2 ml. of 2% sodium hydroxide solution). The mixture was stirred well, diluted to 5 times its volume with water and left to stand in the cold for 24 hr. After filtration, the solution was treated with 5 ml. of 20% sodium sulfide solution and filtered again. It was then concentrated to 40 ml. by vacuum distillation and extracted with 5 successive portions of a chloroform-alcohol mixture (1:1, v./v.). The combined organic layers were dried over sodium sulfate and evaporated to dryness. The brown solid residue (about 1 g.) was crystallized from boiling water to give 450 mg. of a crude crystalline glucoside melting at 220–230°. Further recrystallization from water gave colorless shining platelets of ammajin (II), m.p. 259–260° (dec.), $[\alpha]_D^{25}$ −60° (in 50% ethanol). Yield of the pure glucoside with respect to the seeds: 0.145%. II is sparingly soluble in cold water and alcohol and moderately soluble (about 1 g. in 300 ml.) in these solvents on boiling. It dissolves in glacial acetic acid on heating and is practically insoluble in methanol, acetone, and ethyl acetate. Dilute aqueous solutions of II fluoresce blue in ordinary light; under ultraviolet light, the fluorescence is intense violet-blue. II dissolves in concentrated sulfuric acid and alcoholic potash with a yellow color.

Anal. Calcd. for $C_{20}H_{24}O_9$: C, 58.81; H, 5.92. Found: C, 58.47; H, 6.01.

Acetylation of ammajin. One half gram of II was refluxed for 2 hr. with 15 ml. of acetic anhydride and 1 g. of anhydrous sodium acetate. The cooled mixture was decomposed with ice and water, and the colorless precipitate which separated out was crystallized from dilute ethanol to give 450 mg. of ammajin tetraacetate, m.p. 227°.

Anal. Calcd. for $C_{28}H_{32}O_{13}$: C, 58.31; H, 5.60. Found: C, 58.31; H, 5.71.

Hydrolysis of ammajin. One-half gram of II was refluxed for 2 hr. with 25 ml. of 5% hydrochloric acid. The precipitated aglucone (I) was collected and crystallized from benzene as colorless rods, (250 mg.) m.p. 189–190°; $[\alpha]_D^{25}$ +25° (in chloroform) [reported for marmesin: m.p. 189.5°; $[\alpha]_D^{25}$ +26.8° (in chloroform)[4]]. Addition of I to an authentic sample of marmesin from *Aegle marmelos* Correâ did not depress its melting point. I was soluble in chloroform, moderately soluble in alcohol and acetone, sparingly soluble in benzene, and practically insoluble in petroleum ether and water. Very dilute aqueous or alcoholic solutions of I had a strong blue-violet fluorescence in ultraviolet light.

The ultraviolet spectrum of I (0.008 mg./ml. in ethanol, 25°) showed a characteristic peak at 337 mμ and a much less sharp maximum at 260 mμ, with minima at 245 and 266 mμ. The R_f value of I (ascending paper chromatography; water as a solvent, temp. 30°) was 0.65 (reported for marmesin: 0.66[8]). The untreated spot appeared violet-blue under ultraviolet light (and silvery blue after spraying with dilute sodium hydroxide solution).

Anal. Calcd. for $C_{14}H_{14}O_4$: C, 68.30; H, 5.74; m.wt., 246. Found: C, 68.15; H, 5.83; equivalent weight (by titration): 249.

Acetylation of I with acetic anhydride–sodium acetate gave a monoacetyl derivative, m.p. 132° (from alcohol) (reported for marmesin acetate: 130°[4]).

Anal. Calcd. for $C_{16}H_{16}O_5$: C, 66.66; H, 5.55; m.wt., 288. Found: C, 66.52; H, 5.61; equiv. wt. (by titration): 142.

When a mixture of I and phosphorus pentoxide was either heated as such in a sublimation apparatus,[9] or refluxed in benzene, a dehydrated compound, m.p. 138–138.5° was obtained (reported for desoxyoreoselone obtained by the dehydration of nodakenetin: 138–139°[9] and of marmesin: 138–140°[4]).

Anal. Calcd. for $C_{14}H_{12}O_3$: C, 73.69; H, 5.26. Found: C, 73.8; H, 5.12.

By refluxing with dilute sodium hydroxide in the presence of mercuric oxide, I was converted to the corresponding *trans*-coumaric acid, m.p. 204–205° (dec.) (reported for *trans*-marmesic acid: 204° dec.[4]).

Oxidation of I with chromic acid gave pale yellow needles, m.p. 258–262° (dec.) having a violet ferric chloride reaction in alcohol (reported melting point for umbelliferone-6-carboxylic acid from marmesin: 260°[4]).

Bromination of II with one equivalent of bromine in alcoholic or acetic acid medium, at room temperature, gave colorless shining plates of a monobromide, m.p. 230–231°.

Anal. Calcd. for $C_{14}H_{13}O_4Br$: C, 51.71; H, 4.03; Br, 24.58. Found: C, 51.58; H, 3.93; Br, 25.32.

Identification of the sugar part of ammajin. The aqueous filtrate from I (see under "Hydrolysis of Ammajin") was extracted with chloroform, neutralized with sodium bicarbonate, treated with sodium acetate (2 g.) and phenylhydrazine hydrochloride (0.5 g.), and the mixture was heated on a water bath for 1 hr. The yellow precipitate which crystallized out on cooling had m.p. 204–205°, after recrystalliza-

(4) A. Chatterjee and S. S. Mitra, *J. Am. Chem. Soc.*, 71, 606 (1949).

(5) J. Arima, *J. Chem. Soc. Japan*, 48, 88 (1927).

(6) Comp. E. Späth and E. Tyray, *Ber.*, 72, 2089 (1939).

(7) All melting points are uncorrected. Elementary microanalyses by Dr. A. Bernhardt, Mülheim, Germany. Determination of ultraviolet spectra and optical rotations by courtesy of Dr. M. F. Messeid, Cairo.

(8) D. P. Chakraborty and P. K. Bose, *J. Indian Chem. Soc.*, 33, 905 (1956).

(9) E. Späth and P. Kainrath., *Ber.*, 69, 2062 (1936).

tion from alcohol (undepressed by an authentic sample of D-glucose osazone).

Partial synthesis of ammajin. Marmesin (I) (obtained by the hydrolysis of II) (0.30 g.), β-pentaacetyl-D-glucose (0.5 g.) and 10 mg. of *p*-toluenesulfonic acid were mixed and heated in a test tube at 130–135° for 1 hr. The dark melt was warmed with 5 ml. of benzene and filtered. The filtrate was then treated, while warm, with isoheptane, until a precipitate appeared. The mixture was cooled in ice and the clear supernatant liquid was decanted from the yellow resinous product sticking to the bottom of the flask. The solution was warmed and treated again with isoheptane to incipient turbidity, and left in the cold overnight. The almost colorless product which separated out was crystallized from dilute ethanol twice to give 100 mg. of colorless needles, m.p. 225–227°, giving no depression with a sample of the tetraacetate prepared from natural ammajin.

The synthetic acetate (250 mg.) was hydrolyzed with dry ammonia in methanol[6] to give 120 mg. of ammajin, identified by its melting point (258–260°) and its mixed melting point (no depression) with the natural glucoside obtained from the plant.

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