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ISOLATION OF VISNAGAN, THE AMORPHOUS CORONARY-DILATOR PRINCIPLE OF *AMMI VISNAGA*

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A considerable amount of chemical and biological work has been reported within the past few years involving the crystalline principles of the seeds of *Ammi visnaga*. Only brief reports have appeared describing a crude amorphous fraction, designated as visnagan, which is claimed to have coronary-dilator properties (1, 2).[2]

Samaan described visnagan as a dark, oily liquid distilling with decomposition at 165° at 20 mm. and obtained in about 2% yield from the seeds. Similar material has been prepared which upon further purification has yielded a colorless, hard glass which did not distill at 10^{-6} mm. at 120°. This constitutes somewhat less than half of the crude visnagan preparation. Analyses on a number of preparations indicate a molecular formula of $C_{20}H_{27-28}O_7$ for visnagan. It formed no derivatives which would indicate the presence of hydroxyl, carbonyl or carboxyl groups and contains no methoxyl groups. Visnagan is optically active, $[\alpha]_D^{25}$ +30.5° ±0.5° (16 mg. per ml. in dioxane). Although existing as a hard glass at 25°, the principle is sufficiently fluid at 60° to allow a refractive index determination; n_D^{60} 1.5345. Mild alkaline hydrolysis of visnagan yields one acidic group, stronger hydrolysis yields nearly two. The principle reacts with one mole of iodine bromide (Hanus solution).

Ultraviolet absorption spectra[3] (Fig. 1) show an absorption maximum of $E_{1\,cm}^{1\%} = 308.2$ at λ 325 mμ and a minimum of $E_{1\,cm}^{1\%} = 63.8$ at λ264 mμ indicating the possible presence of an aromatic ring conjugated to another unsaturated system. Infrared absorption data[4] (Table I) suggest the presence of a *para*-substituted phenyl group, methyl groups, a bonded -OH group, and perhaps a strained ring carbonyl but no —COO-group.

[1] Present address: Smith, Kline, and French Laboratories, Philadelphia, Pa.

[2] Preliminary tests by Dr. F. P. Luduena of these laboratories indicate that visnagan is approximately of the order of activity of aminophylline in decreasing the depressor action of Pituitrin® on the dog heart. It appears to be approximately twice as active as khellin as a coronary dilator in perfusion experiments. Visnagan is quite insoluble in water but a turbid solution for intravenous administration in animals may be prepared by dissolving the material in a small volume of ethanol and diluting to about 0.5 mg. per ml. with water.

[3] The ultraviolet absorption spectrum was obtained with a Cary recording Spectrophotometer, Model 11, Serial 37, slow scanning speed, 50 slit schedule, 10.00-cm. quartz cells, and 95% ethanol as solvent. The data were plotted as molar extinction coefficients, using an average molecular weight figure of 403.

[4] Infrared absorption spectra were measured by Dr. John H. Harley at Rensselaer Polytechnic Institute with 0.025-mm. sections using a Perkin-Elmer Model 12B Recording Spectrophotometer. In the high frequency region, a quartz prism was used to obtain greater dispersion than would be possible with the standard rock-salt prism. The data obtained are represented in Table I with intensity of adsorption at various frequencies denoted by numbers ranging from 1 to 5, the higher numbers indicating more intense adsorption.

A crystalline impurity exists with crude visnagan which is difficult to separate completely and which may appear as crystals after long standing of the resin. This product melts slowly between 133–140°; the apparent formula is $C_{15}H_{12}O_5$. It forms a crystalline salt with hydrogen chloride in ether as do the *gamma*-chromone principles. Visnagan does not form an oxonium salt. The crystalline principle has no coronary-dilator properties.

EXPERIMENTAL

Isolation of visnagan. A concentrate containing visnagan was prepared essentially according to the procedure of Samaan (1). The oil was dissolved in ether and then diluted with a petroleum solvent (Skellysolve B) until no further precipitate of visnagan was formed.

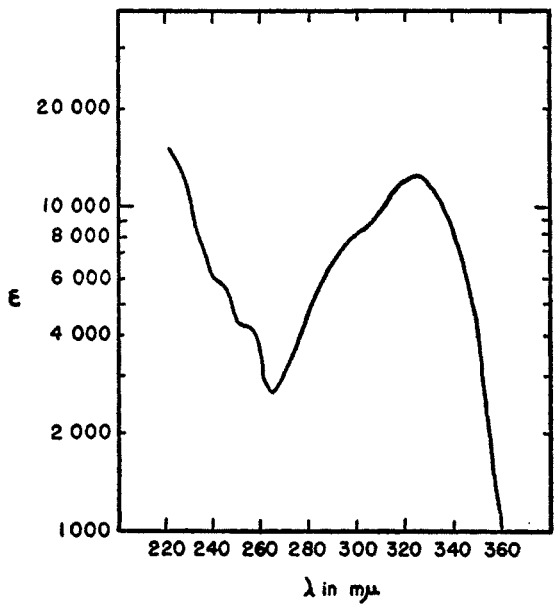

FIGURE 1. ULTRAVIOLET ABSORPTION SPECTRUM OF VISNAGAN

This procedure was repeated several times and served to separate the soluble lipids from visnagan which is insoluble in petroleum solvents. The insoluble resin was dissolved in dry ether and ethereal hydrogen chloride was added. The crystalline precipitate (I) was filtered off and Skellysolve B was added slowly with shaking to the ether solution to precipitate a tacky, dark tar. The solution was decanted occasionally from the black tar and more Skellysolve added until the material which separated was a clear resin. The dark tar was discarded and the clear resin was completely precipitated from solution. The resin was dissolved in ether, diluted with Skellysolve B to the point of incipient turbidity, and then passed over a column of silica gel[5] previously wet with ether. Considerable visnagan and some crystalline impurity (I) appeared in the filtrate after development with ether. The column was eluted with an ethanol-ether solution and the slightly colored, hard glass-like visnagan was obtained upon evaporation of the eluate. The remainder of the color was removed by passing a dioxane solution of the resin over alumina (Brockmann, Merck) and evaporation of the filtrate.

[5] Activated, 28–200 mesh from the Davison Chemical Corporation.

Anal. Found: C, 65.65; H, 7.04; Another prep., C, 65.65; H, 6.68. Cryoscopic Mol. wt. determination in dioxane, 387.

Calc'd for $C_{22}H_{28}O_7$: C, 65.33; H, 6.98; Mol. wt. 404.4.

Calc'd for $C_{22}H_{26}O_7$: C, 65.66; H, 6.52; Mol. wt. 402.4.

Crystalline impurity from visnagan. The precipitate I obtained from the ethereal hydrogen chloride solution was treated with water to decompose the salt and the product was recrystallized about six times from water; m.p. 133–140° (corr.).

TABLE I

INFRARED ABSORPTION OF VISNAGAN

FREQUENCY, CM.$^{-1}$	INTENSITY	GROUP ASSIGNMENTS
666	–	—COO—
759	2	
774	3	*para*-substitution
840	5	
875	4	
889	3	usually phenyl
922	3	" "
948	1	" "
965	1	" "
991	1	" "
1007, 1028	5	
1042	4	—OH
1115	5	*para*-substitution
1144	5	
1185	3	*para*-substitution
1227	5	
1281	3	
1352	3	$-CH_3$
1373	4	$-CH_3$
1402	3	
1439	2	$-CH_3$
1462	3	$-CH_3$
1496	3	$-CH_3$, phenyl
1603	5	phenyl
1642	2	
1765	5	
2670	1	
2925	5	$-CH_3$
3460	2	bonded —OH

Anal. Found: C, 66.20; H, 4.33; Cryoscopic Mol. wt. in dioxane, 260.

Calc'd for $C_{15}H_{12}O_5$: C, 66.17; H, 4.44; Mol. wt., 272.3.

Tests for functional groups in visnagan. Visnagan in ethanol-water solution is neutral and does not react with 0.1 *N* NaOH at 25°; heating with a 0.1 *N* NaOH solution at 100° leads to formation of from one to two acidic groups depending upon length of time of heating (10 to 60 minutes).

Visnagan was recovered unchanged after treatment with phenylhydrazine or hydroxylamine under the usual conditions. *p*-Nitrobenzoyl chloride in pyridine did not react and no derivative with phenyl isocyanate could be obtained. Visnagan decolorized bromine water and reacted with one mole of iodine bromide in acetic acid.

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SUMMARY

Visnagan, the amorphous coronary-dilator principle of *Ammi visnaga* seeds, has been obtained as a hard glass and assigned the tentative molecular formula of $C_{20}H_{26-28}O_7$.

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REFERENCES

(1) SAMAAN, *Quart. J. Pharm. Pharmacol.*, **4**, 14 (1931); *Quart. J. Pharm. Pharmacol.*, **6**, 13 (1933).

(2) SAMAAN, *Quart. J. Pharm. Pharmacol.*, **18**, 82 (1945).