ISOLATION AND IDENTIFICATION OF A CANNABINOID-LIKE COMPOUND FROM *AMORPHA* SPECIES

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ABSTRACT.—The petroleum ether extracts from several plants have been screened on tlc plates with the chromogenic reagent Fast Blue B. salt. Extracts from different plant parts of *Amorpha fruticosa*, *A. nanna*, and *A. canescens* gave several spots with color and R_f values similar to those of *Cannabis sativa* extract. The major compound, amorphastilbol (**1**), was isolated by column chromatography, purified, and characterized by mp, elemental analysis, uv, ir, mass, pmr, and cmr spectroscopy. Further information was obtained by analyzing the oxidation products of the compound. The evidence indicates that amorphastilbol is a phenolic stilbene terpenoid with a molecular formula of $C_{24}H_{28}O_2$ and structural formula: (*E*,*E*)-2-(3',7'-dimethyl-2',6'-octadienyl)-5-(2-phenylethenyl)-1,3-benzenediol. This is the first report of the isolation of a naturally-occurring stilbene derivative possessing a terpenoid moiety.

Confirmation of the identification of *Cannabis sativa* L. and its preparation hashish is necessary before criminal justice can be administered. Identification and confirmation by forensic laboratories are usually carried out by microscopic examination coupled with chemical tests. Several simple tests are available (1, 2) and are considered specific. The application of the chromogenic reagent, Fast Blue B. salt (FBB) to a petroleum ether extract or to the powder to be investigated has been reported (3, 4). Sometimes, any color obtained is mistaken for a positive result. To resolve the problem of false positives, Forrest and Heacock (5) used tlc coupled with FBB visualization. Hughes and Warner (6) reported that there is no compound or mixture of compounds that coincidentally chromatographs and develops the same colors as cannabinoids.

In an initial screening of the petroleum ether extracts of different plants by tlc and FBB, *Amorpha* species (Leguminosae) gave chromatographs with close resemblance to *Cannabis* extract, but failed to give a positive modified Duquenois test (2).

A research of the literature revealed that several rotenoids have been isolated from chloroform-methanol extracts of different *Amorpha* sps. (7, 8, 9). However, no phytochemical analysis has been carried out to determine the nature of the petroleum ether extractables.

In this study we are reporting the isolation, characterization and chemical structure of the major compound in the petroleum ether extract of the leaves which produces a reddish-orange color with FBB.

EXPERIMENTAL

PLANT MATERIAL.—The above-ground parts of the wild-growing plants *Amorpha nana* (Nutt.) and *Amorpha canescens* (Pursh) were collected in the flowering or fruiting stage from the State of North Dakota. The plants were identified by the late Dr. O. A. Stevens, Professor Emeritus of Botany at North Dakota State University. Herbarium specimens are on deposit in the Department of Pharmacognosy, NDSU. *Amorpha fruiticosa* (L.) was obtained from New Jersey through Dr. L. J. Schermeister, Professor of Pharmacognosy, NDSU. The fresh plant material was separated into the respective plant parts and air dried before grinding.

ANALYTICAL TECHNIQUES.—The melting point was determined on Arthur-Thomas melting point apparatus and is reported uncorrected.

Elemental analysis was performed by Galbraith Labs. Inc., Knoxville, Tenn. The mass spectrum was recorded on a Varian CH 5. The ultraviolet spectra were recorded on a Varian Cary 118. The ir spectrum (KBr disc) was obtained with a Perkin-Elmer 167 spectrophotometer, and only the main peaks are cited. Pmr spectra ($CDCl_3$ and D_2O) were obtained with a Varian T60 and EM 390. Cmr spectra ($CDCl_3$) were obtained with a JEOL FX-60 spectrometer; a 20 to 45° flip angle and 6000–50,000 transients were used, depending on the compound and the nature of the experiment. Chemical shifts are reported in ppm downfield from tetramethylsilane. The compound was oxidized (10), and the products were identified by hplc (Waters Associates Model 202). A reverse phase cyanopropysilane column with a liquid phase consisting of 80% acetonitrile and 20% water were used. Tlc (Silica gel G 0.25 mm plates) was used also for the identification of the oxidation products.

EXTRACTION AND PRELIMINARY PURIFICATION.—A 700 gm sample of powdered *A. nana* leaves was extracted with petroleum ether (bp 35–60°) on a continuous extraction apparatus. The extract was evaporated with a flash evaporator. The concentrated extract was extracted with 2% aqueous sodium hydroxide (5 x 200 ml). The combined sodium hydroxide fraction was then acidified with concentrated hydrochloric acid and backextracted with petroleum ether (5 x 200 ml). The combined petroleum ether was washed with distilled water (3 x 10 ml), and the organic layer was filtered through anhydrous sodium sulfate. The filtrate was then concentrated to a minimum volume, mixed with silica gel, air-dried, and subjected to column chromatography.

ISOLATION AND IDENTIFICATION.—The dried silica mixture was applied to a column (1 x 30 in) packed with silica gel (40–140 mesh). Elution was effected with solvent systems of increasing polarity in the following order: petroleum ether, 1% ether-petroleum ether, 2% ether-petroleum ether and 5% ether-petroleum ether collecting 40 ml fractions. Elution with the last system was continued till a negative FBB test was obtained. The different fractions were evaporated and combined according to tlc analysis (chloroform-petroleum ether (60:40) as a solvent). The fractions which showed a spot with R_f 0.36 were pooled and evaporated. The resulting residue (995 mg) was recrystallized from ether-hexane twice; the yield was 196 mg (.03%). The crystals obtained were tested for purity by tlc and hplc.

Samples of other plant parts were extracted as above, and the yield was much less. *A. fruticosa* gave less yield, and *A. canescens* yielded only a trace amount.

The isolated compound is a white crystalline substance mp 94.5–95°, uv λmax (absolute EtOH) 316 nm (log ε 4.46), 238 nm (log ε 4.19); λmax (0.1N NaOH) 322 nm (log ε 4.25), 262 nm (log ε 4.11), 230 nm (log ε 4.15). No shift was observed in 0.1N HCl. The ir spectrum (KBr), 3360–3400 cm^{-1} (O–H stretch), 1605 cm^{-1}, 1575 cm^{-1} (C=C, aromatic).

Elemental analysis, Found: C, 82.77%, H, 8.26%; Calculated for $C_{24}H_{28}O_2$: C, 82.84%, H, 8.11%. Mass spectrum (70 ev) *m/e* (relative intensity): 348 (47%, M^+), 333 (2.4%, M^+–15), 305 (4.7%, M^+–43), 279 (28.4%, M^+–69), 265 (25.5%, M^+–83), 225 (100%, M^+–123).

RESULTS AND DISCUSSION

The spectral similarities (11, 12, 13, 14) between amorphastilbol (**1**) and cannabigerol[1] (**2**) figure 1, particularly between the pmr spectra and especially the base peak at *m/e* 225 in the mass spectrum, strongly support a partial structure having a 1,3-benzenediol ring substituted with a dimethyl-2,4-octadienyl aliphatic side chain. The pmr spectrum of amorphastilbol (table 1) showed an absence of peaks at δ 2.44, 1.32 and 0.88 that are present in **2**, due to the pentyl aliphatic side chain. The proton benzylic doublet at δ 3.45 in (**1**) was decoupled by irradiation of the vinyl proton at δ 5.2 consistent with its assignment as C–1′. The protons of the vinyl methylene groups at C–4′ and C–5′ were a narrow multiplet at δ 2.03. Decoupling of the C–6′ vinyl proton at δ 5.1 supported this assignment. The principle mass spectral peaks are readily assigned to fragments of a dimethyl-2, 6-octadienyl side chain (figure 2), confirming the structural assignment of this group. The C–2′ double bond in cannabigerol has been investigated previously, both spectroscopically and synthetically, and has been found to have *E* stereochemistry (12, 13, 14). This unequivocally establishes the stereochemistry of the C–2′ double bond in amorphastilbol as *E*, also, based on the δ 3.45 chemical shift

[1]Applied Science Laboratories Inc., State College, Penn.

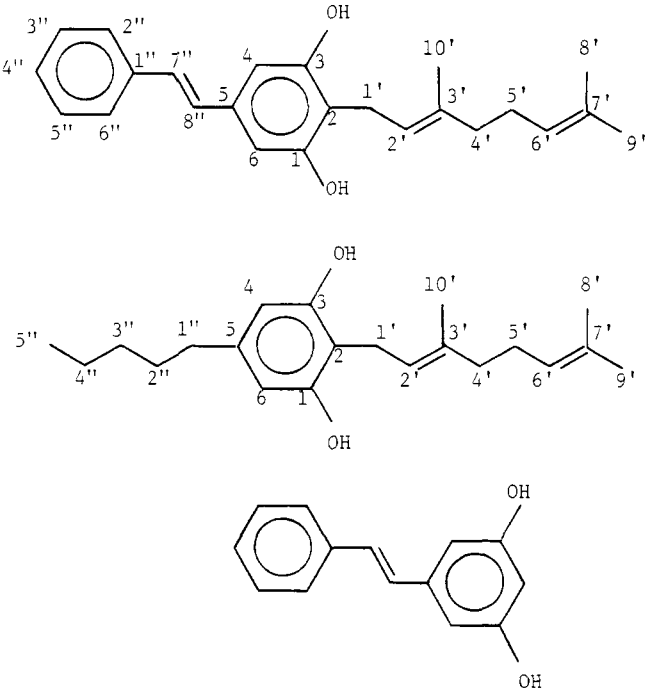

FIG. 1. Structures of amorphastilbol (**1**), cannabigerol (**2**), and pinosylvin (**3**).

for the C–1' methylene group in the pmr spectrum. The cmr spectrum (table 2) also supports this assignment. The 1,3-benzenediol ring, alkyl substituted at the 2 and 5 positions, was readily apparent in the cmr spectrum where the characteristic peaks matched closely those present in cannabigerol and Δ 9 tetrahydrocannabinol (15) (table 2). The differences observed between **1** and **2** for carbons 4 and 6 are

TABLE 1. Pmr spectra.

Proton(s) on Carbon Number	Amorphastilbol (**1**)			Cannabigerol (**2**)		
	Chemical Shift (ppm)	Area	Multiplicity	Chemical Shift (ppm)	Area	Multiplicity
1, 3	5.05	2	s	5.05	2	s
4, 6	6.55	2	s	6.18	2	s
1'	3.45	2	d, J=6 Hz	3.36	2	d, J=6 Hz
2'	5.20	1	t, J=6 Hz	5.22	1	t, J=6 Hz
4', 5'	2.03	4	m	2.03	4	m
6'	5.10	1	m	4.94	1	m
8'	1.62	3	s	1.57	3	s
9'	1.68	3	s	1.75	3	s
10'	1.76	3	s	1.78	3	s
1"				2.44	2	t
2"–4"	7.2–	5	m	1.32	6	m
5"	7.45			0.88	3	t
6"						
7", 8"	6.98	2	s			

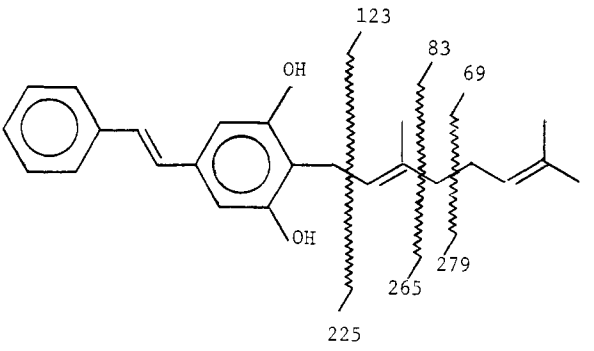

FIG. 2. Mass spectral fragmentation of amorphastilbol.

consistent with the different 5-substituent in (**1**) relative to (**2**). Carbon-2 in cannabigerol could not be uniquely identified under the pulsing conditions used, due to its low signal intensity.

The remaining portion of the structure, C_8H_7, must have five double bond equivalents. This is consistent with one phenyl ring and a carbon-carbon double bond and is supported by the appearance of peaks of δ 6.98 and 7.2–7.45 with an area of 7 protons in the pmr spectrum of (**1**) which are not present in the cannabigerol spectrum. The presence of a monosubstituted phenyl ring is consistent

TABLE 2. Cmr spectra.

Carbon	Amorphastilbol (**1**)			Cannabigerol (**2**)	Δ^9 Tetrahydrocannabinol
	Chemical Shift (ppm)[a]	Area	Multiplicity	Chemical Shift (ppm)	Chemical Shift (ppm((15)
1, 3	155.1	2	s	154.7	154.4, 154.1
2	113.2	1	s	110.5[b]	110.4
4, 6	106.5	2	d, J=163 Hz	108.3	107.5, 109.8
5	136.8	1	s	142.6	142.5
1'	22.5	1	t, J=127 Hz	22.5[b]	33.6
2'	123.6	1[b]	d, J=146 Hz	123.7	123.7
3'	139.4	1	s	138.8	133.8
4'	39.7	1	t[b]	39.7	31.1
5'	26.4	1	t, J=124 Hz	26.4	25.0
6'	121.2	1[b]	d, J=159 Hz	121.4	45.7
7'	132.1	1	no[c]	131.9	77.1
8'	17.7	1	q, J=119–128 Hz	17.7	
9'	25.6	1	q, J=119–128 Hz	25.7	
10'	16.2	1	q[b]	16.2	
1"	137.1	1	s	35.5	35.4
2"	126.4[b]	2	d	30.7	30.5
6"				31.4	31.4
3"	128.6[b]	2[b]	d, J=140–160 Hz	22.3[b]	22.5
5"				14.0	14.0
4", 7", 8"	127.5[b]	1	d		
	128.0[b]	1	d		
	128.6[b]	1	d		

[a]Assignments made with the aid of off resonance and selective decoupling.
[b]These assignments, integrations or coupling constants are in doubt and are made on the basis of anlogy or another data.
[c]Not observable.

with the oxidation of (**1**) to benzoic acid. Support for a 5-styryl group on the 1,3-benzenediol ring came from the uv spectrum of (**1**). The features of the nonconjugated 1,3-benzenediol absorption of (**2**) also present in cannabidiol (11, 16) are largely unaffected by the nonconjugated 2 and 5 substituents. In amorphastilbol, however, the conjugation of a styryl moiety changes the basic 1,3-benzenediol absorption to longer wavelength and increases the absorbtivity considerably. This absorption is in accordance with that of *E*-stilbene (17, 18) and of pinosylvin (**3**), which has an *E*-stereochemistry. The uv absorption of pinosylvin has variously been reported to be from 305 to 312 nm (19, 20, 21). The shift of 4 nm (312 to 316 nm) on addition of base to pinosylvin (20) compared to 6 nm (316 to 322 nm) for amorphastilbol proves that the hydroxyl groups must be in a 1,3 orientation relative to the substituent double bond. A 1,2 or 1,4 orientation would cause a shift of 20 to 30 nm (20). The observed maximum of 316 nm for amorphastilbol strongly favors *E*-stereochemistry.

The vinyl pmr absorption for pinosylvin occurs at δ 7.05 compared to δ 6.98 for amorphastilbol. A *Z*-stilbene would be expected to absorb at higher field, thus supporting *E*-stereochemistry. The cmr spectrum further favors this assignment. By substracting the cmr spectrum of cannabigerol or tetrahydrocannabinol from that of amorphastilbol, the peaks due to the styryl moiety became evident as a cluster of five peaks from 126.4 to 137.5 ppm integrating for 8 carbons. While unequivocal assignments cannot be made without labeling studies (table 2), the general intensities and positions of the peaks resemble those of *E*- rather than *Z*-stilbene, which are taken for comparison. The fact that the *Z*-stilbene vinyl carbons absorb at 130.1 ppm (established by selective decoupling experiments) while neither *E*-stilbene nor amorphastilbol absorb at that particular chemical shift supports the *E*-stereochemistry for the stilbene moiety of (**1**).

Thus, the structure of amorphastilbol may be assigned as (*E,E*)-2'-(3',7'-dimethyl-2,6-octadienyl)-5-(2,phenylethenyl)-1,3-benzenediol, as shown (figure 2).

The isolation of amorphastilbol could be of considerable importance from biosynthetic viewpoint; not only does it confirm the presence of stilbene derivatives in leguminous plants, but it also shows a possible link between cannabinoids and other plant constituents. The latter is supported by the recent report of the isolation of dihydrostilbenes from Thailand Cannabis (22).

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