films will be compared with literature data on free films in order to evaluate the utility of the model.

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Monomolecular films-polymeric film formers Surface pressure-area isotherms-determination

Compression---film effect

Lipids from the Seeds of Abrus precatorius By MORTON S. LEFAR, DAVID FIRESTONE, EUGENE C. COLEMAN,

NATHAN BROWN, and DONALD W. SHAW

The lipids obtained from Abrus precatorius were separated into a saponifiable and unsaponifiable fraction. Analysis of the saponifiable fraction by gas-liquid chromatography (GLC) showed the presence of a series of C-8 to C-24 saturated and C-16 to C-24 unsaturated acids. Column chromatography of the unsaponifiable material yielded a hydrocarbon, alcohol-methyl sterol, and sterol fraction. GLC showed that the hydrocarbon fraction contained squalene, a homologous series of C-16 to C-35 normal, iso and/or anteiso, and possibly multibranched compounds. The alcoholmethyl sterol fraction contained a series of C-14 to C-28 normal alcohols in addition to β -amyrin and cycloartenol. Stigmasterol, β -sitosterol, and campesterol were the major sterol constituents identified by GLC.

brus precatorius (also called Jequirity, Gunja, A Kunch, and Rati) is a perennial twiner belonging to the family Leguminosae that grows throughout India and other tropical countries (1). It contains some of the most lethal plant poisons in the tropics. Many children have been attracted to its color and have accidentally swallowed the seed. Jequirity beans are frequently used as eyes in dolls, toys, necklace beads, and clothing ornaments. Some of the preparations from this plant induced severe cytotoxicity in concentrations of less than 0.01 mcg./ml. of solid material. Extracts of Abrus precatorius initially suppressed the growth of cancer cells and prolonged the survival of laboratory animals (2).

The biological effects of extracts of the seed have stimulated the need for further information concerning the identity of the compounds contained in Abrus precatorius.

EXPERIMENTAL

Source and Preparation of Material-Ripe seeds of Abrus precatorius were obtained from Herbst Brothers, Seedsmen, Inc., Brewster, N. Y. Approximately 65 g. of seeds dispersed in powdered dry ice were ground to a powder in a Wiley mill.

Extraction of Seeds-The powdered seeds were extracted successively with two 200-ml. portions of n-hexane, ethyl ether,¹ and distilled water at room temperature, and finally with two 500-ml. portions of 95% ethyl alcohol in a Soxhlet for 6 hr. Evaporation of 250 ml. of the hexane and ether fractions yielded, respectively, 372 and 177 mg. of yellow oils.

Saponifiable and Unsaponifiable Fractions-The saponifiable and unsaponifiable fractions were extracted (3). The alkaline soaps obtained were acidified with 1 + 1 HCl, extracted three times with petroleum ether, and then converted to the methyl esters (4).

Gas-Liquid Chromatography of Methyl Esters²-The mixture of methyl esters was subjected to GLC, using a Wilkins Aerograph model 1520 instrument fitted with a flame-ionization detector The 2.7 m. \times 0.6 cm. i.d. (9 ft. $\times \frac{1}{4}$ in.) aluminum column was packed with 20% polydiethylene glycol

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¹The ether and hexane extracted residues did not inhibit the growth of diploid cells (Hayflick strain, Wistar 138; obtained from Baltimore Biological Laboratories, Cockeys-

³ Stationary phases, packings, and standards were obtained from Applied Science Laboratories, State College, Pa.

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TABLE I—UNSAPONIFIABLE COMPONENTS OF Abrus precatorius

Fraction	Ethyl Ether Extract 2.2 mg.	Hexane Extract 18.4 mg.
Hydrocarbon	C16-C24	C15-C25
,	Squalene, C ₂₁ OH–C ₃₂ OH	C27-C35
Alcoholmethyl	C20OH-C28OH	C ₁₀ OH-C ₂₈ OH
sterol	C ₂₉ OH, C ₃₃ OH (?)	β-Amyrin
	Campesterol (?) β-Amyrin Cycloartenol	Cycloartenol
Sterol	Cholesterol	Cholesterol
	Brassicasterol	Brassicasterol
	Campesterol	Campesterol
	Stigmasterol	Stigmasterol
	B-Sitosterol	8-Sitosterol
	Methyl sterols (?)	Methyl sterols

 TABLE II—PERCENT OF TOTAL FATTY ACIDS^a

 EXTRACTED FROM Abrus precatorius

Fatty Acid	Hexane Fraction, %	Ether Fraction, %
Octanoic (8:0)	trace	
Dodecanoic (12:0)	trace	0.2
Tetradecanoic (14:0)	0.1	0.7
Hexadecanoic (16:0)	4.0	10.5
9-Hexadecenoic	0.2	1.3
Unidentified ^b	0.2	0.7
Octadecanoic (18:0)	1.0	1.9
9-Octadenenoic (18:1)	23.0	22.3
9,12-Octadecadienoic (18:2)	7.7	30.7
Eicosanoie (20:0)	0.6	
11-Eicosenoic (20:1)	5.2	10 0
9.12.15-Octadecatrienoic	10.6	16.8
(18:3)	,	
Docosanoic (22:0)	3.9	2.2
13-Docosenoic (22:1)	33.6	3.4
Tetracosanoic (24:0)	1.9	9.4
15-Tetracosenoic (24:1)	2.9	
Unidentified	4.8	_

^a As methyl esters; not corrected for detector response. ^b Retention time versus 16:0 = 1.47. ^c Retention time versus 16:0 = 9.07.

succinate (DEGS) on 60–80 mesh Chromosorb W. The gas chromatographic conditions were as follows: helium gas flow 70 ml./min.; column temperature 207°; injection port 253°; cell temperature 268°.

Identification of Esters—The retention times of the sample components were compared with that of highly purified esters on polar (DEGS) and nonpolar columns (2.7 m. \times 0.6 cm. i.d.; 3% SE-30 on 60-89 mesh Chromosorb W). Semilogarithmic plots of retention times *versus* carbon number were used to identify 15-tetracosenoic acid and to confirm assignments.

Column Chromatography of Unsaponifiables— Magnesium silicate³ was heated to 260° for 2 hr., then deactivated with 10 g. of water per 100 g. of magnesium silicate and allowed to equilibrate overnight. The 18.4 mg. and 2.2 mg. of unsaponifiable material from the hexane and ether fractions, respectively, were chromatographed on a 2.5×30 cm. column containing 20 g. of the prepared magnesium silicate. The hydrocarbons were eluted with 40 ml. of 10% anhydrous ethyl ether in hexane and the alcohol-methyl sterols with an additional 140 ml. of the same mixture. The sterols were then eluted with 150 ml. of 50% ether in hexane.

Gas-Liquid Chromatography of the Hydrocarbon, Alcohol-Methyl Sterol, and Sterol Fraction—The three fractions eluted from the column were subjected to GLC on a Barber-Colman model 5000 instrument utilizing the hydrogen-flame detector. The instrument was fitted with a 1.8 m. \times 0.6 cm. i.d. (6 ft. \times ¹/₄ in.) glass column filled with 3% JXR on 100–120 mesh Gas Chrom Q. Nitrogen was used as the carrier gas at 30 p.s.i. Detector and sample temperatures were maintained at 262°. The column temperatures were controlled at 180° and 240° (see Table I). For quantitative estimation of various components, areas beneath gas chromatographic peaks were determined by the peak height retention time method (5).

RESULTS AND DISCUSSION

The fatty acids detected in *Abrus precatorius* in this study differ from an earlier chemical investigation (1) of the lipid fraction which did not report the presence of trace amounts of octanoic (8:0), dodecanoic (12:0), or tetradecanoic (14:0) acids, in addition to larger amounts of 9-hexadecenoic (16:1) acid (see Table II).

GLC revealed that 13-docosenoic (22:1) acid was the third most prevalent component in this preparation. However, Mandiratta and Dutt (1) did not indicate the presence of this lipid.

Examination of the chromatogram of the hydrocarbon fraction and plots of retention time versus carbon number (Fig. 1) indicate the presence of three homologous series in addition to squalene. The first series consists of normal straight chain hydrocarbons in the range $C_{16}-C_{26}$. The second series consists of iso and/or anteiso hydrocarbons (e.g., iso = 2-methyl; anteiso = 3-methyl). Pre-

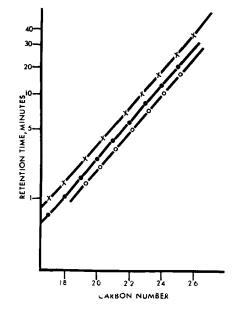


Fig. 1—Semilogarithmic plot of carbon number versus retention time of hydrocarbons obtained from hexane extract. Key: •, iso/anteiso; O, multibranched; ×, normal.

^a Florisil PR, Floridin Co., Pittsburgh, Pa.

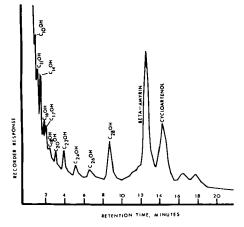


Fig. 2—Chromatogram of alcohol-methyl sterols from hexane extract.

TABLE III-PERCENT" OF TOTAL STEROLS EXTRACTED FROM Abrus precatorius

	Hexane	Ether
Sterol	Fraction, %	Fraction %
Cholesterol	1.3	2.3
Brassicasterol	2.8	1.7
Campesterol	21.0	16.5
Stigmasterol	46.8	50.8
β -Sitosterol	24.8	26.9
Unidentified ^b	2.4	1.8
Unidentified ^c	0.9	<u> </u>

^a Not corrected for detector response. ^b Retention time *versus* cholesterol = 1.84. ^c Retention time *versus* cholesterol = 2.22.

vious examination of standards indicated that iso and anteiso hydrocarbons with the same molecular weights could not be resolved. Iso and anteiso compounds have been reported to be present in vegetable and animals oils (6-9). The third series probably consists of multiple-branched hydrocarbons, which Mold et al. (6) have reported to be present in tobacco leaf wax.

The alcohol-methyl sterol fraction contained mainly a series of C_{10} - C_{28} normal alcohols, β -amyrin, and cycloartenol (Table I and Fig. 2). Campesterol and the C29 and C33 alcohols may be present as minor components.

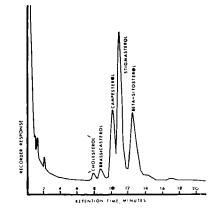


Fig. 3—Chromatogram of sterols from hexane extract.

GLC of the sterol fraction (Table III and Fig. 3) indicated the presence of at least seven components, which were identified by comparison with retention times of standards. Stigmasterol was the most prevalent sterol; Mandiratta and Dutt (1) reported only the presence of sitosterol.

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