Gas Chromatographic Identification of Fatty Acids, Fatty Alcohols, and Hydrocarbons of *Convolvulus pluricaulis* (Chois

ABSTRACT

Convolvulus pluricaulis (Chois) (Family: Convolvulaceae) (Hindi: Shankhapushpi), widely grown in the northern part of India, was analyzed for its fatty acids and waxy constituents. Straight chain hydrocarbons (C_{22}-C_{33}), fatty acids (C_{14}-C_{28}), and fatty alcohols (C_{24}-C_{32}) were found in the whole plant 95% aqueous ethanol extract. Hydrocarbons (C_{27}, C_{31}, and C_{33}), fatty acids (C_{14}, C_{16}, and $C_{18:2}$), and alcohols (C_{26}, iso-C_{28}, iso-C_{30}, and C_{32}) were the major components.

INTRODUCTION

Convolvulus species of plants in general, though famous for their medicinal value, have not been chemically explored to any appreciable extent as have the plants of other families. This is particularly true of a species of Convolvulaceae family known as *Convolvulus pluricaulis* (Chois) which is reported in the ancient Indian system of medicine (Ayurvedic system) to have psychotropic properties (1).

C. pluricaulis (Chois) (whole plant) is reported to contain an alkaloid Shankhapushpine ($C_{17}H_{23}ON$) (2), base A ($C_5H_{11}NO_2$) (3), base B ($C_5H_9NO_2$) (3), water-soluble base C ($C_3H_{11}NO_2$) (4), and a steroidal substance ($C_{40}H_{60}O_5$) (4). No exact information about the nature of these compounds is reported in literature. However, all efforts to isolate these compounds by usual methods and the method reported previously by Basu and Dandiya (2) have failed. The authors did get a very faint indication of nitrogen when an appreciable amount of whole plant distilled 95% aqueous ethanol extract was fused with Na and was tested for presence of nitrogen.

Earlier, we reported (5,6) the characterization of a coumarin, namely scopoletin, β-sitosterol, hentriacontane, cetylalcohol, palmitic, linoleic, and myristic acids and D-glucose and maltose from the extract of *C. pluricaulis* (Chois) whole plant. In this communication, we wish to report the isolation and characterization of a number of fatty acids, fatty alcohols, and straight chain hydrocarbons by gas liquid chromatography (GLC).

EXPERIMENTAL PROCEDURES

The dried powdered plant (4.5 kg) was extracted in a soxhlet with 95% aqueous ethanol. The syrupy mass (403 g) was treated with 1% HCl solution and stirred for 4 hr. The insoluble portion was separated and dissolved in ether. The ether-soluble portion was separated, and 8 g of this was used for column chromatography. A part of this was subjected to thin layer chromatography (TLC) (Kiessel gel G, E. Merck, Darmstadt, Germany) using petroleum ether:benzene (65:35) as the solvent mixture and locating the spots on the plate after spraying with 70% H_2SO_4 containing 0.5% $CuSO_4$ followed by heating in an oven at 150 C for 15 min. Five compounds of R_f 0.92, 0.60, 0.20, 0.10, and 0.04 were revealed. The mixture was resolved by column chromatography on alumina.

COLUMN CHROMATOGRAPHY

The ether-soluble residual mass (8 g) was added to the chromatographic column 90 x 5 cm inside diameter after dissolving in $CHCl_3$. It was eluted in 30 ml fractions (Table I). Each fraction was concentrated, and its components were noted by TLC as before. Fractions having identical patterns were combined.

Wax Hydrocarbons

The fractions 1 and 2 were combined and purified by precipitation with benzene and methanol. An IR spectrum showed absorption at (730, 720 cm^{-1}) and no absorption in hydroxyl region. This combined fraction was subjected to GLC using Shimadzn model GC-4 APF gas chromatograph with a column 2 m long x 4 mm inside diameter packed with shimalite W (80-100 mesh) coated with 1.5% OV-1 operating at 250 C. Hydrogen was used as carrier gas with flow rate at 66 ml/min; chart speed was 5 mm/min.

Wax Esters

Fractions 3-13 were combined. IR spectral analysis showed the combined material to be ester in nature with bands at 1735 cm^{-1} (C=O stretching); 1170 cm^{-1} (C-O ester stretching); and 725, 715 cm^{-1} (alkane chain). The total fraction was refluxed with 2 N ethanolic KOH for 2 hr.

TABLE I

Particulars of the Column Chromatographic Fractions

Fraction no.	Eluent	Wt (g)	R_f
1-2	Petroleum ether	1.610	0.92
3-4	Petroleum ether	1.567	0.60, 0.20
5-8	Petroleum ether	0.500	0.10, 0.20
9-13	Petroleum ether	0.310	0.10, 0.20
14-15	Petroleum ether:benzene 90:10	0.035	0.04, 0.10
16-18	Petroleum ether:benzene 75:25	0.100	0.04, 0.10, 0.20
19-23	Benzene:petroleum ether 50:50	2.357	0.04
24-26	Benzene:petroleum ether 50:50	0.250	0.04, 0.10
27-30	Benzene	0.220	0.04 Steak
31-33	Benzene:chloroform 90:10	0.085	0.04 Steak
34-36	Benzene:chloroform	0.024	0.04 Steak
37-40	Benzene:chloroform 50:50	0.124	0.04 Steak
41-43	Benzene:chloroform 50:50	0.021	0.04 Steak
44-50	Chloroform	0.010	0.04 Steak

Ethanol from the reaction mixture was removed under vacuum. The residue was diluted with water and was extracted with ether. The acid salt was treated with dilute acid, and liberated fatty acid was extracted with ether. Fatty acids thus obtained were converted to methyl esters by the usual procedure. The esters then were subjected to GLC (column: 3.04 m long, 2 mm inside diameter, packed with Chromosorb W [60-80 mesh], coated with 20% diethylene glycol succinate; temperature programed from 180-210 C at 2 C/min; chart speed: 12 in./hr; detector: flame ionization).

Wax Alcohols

Fractions 19-23 were combined and crystallized with acetone. An IR spectrum showed the product to be alcoholic in nature with 3310 cm^{-1} (OH stretching); 1055 cm^{-1} (C-O stretching for alcohol); and 730, 720 cm^{-1} (alkane chain). This combined and recrystallized material was analyzed by GLC (column: stainless steel 3 ft x 1/8 in., packed with Chromosorb W, [100-120 mesh] on 15% SE 30; carrier gas: N_2 with a flow rate 24 ml/min; chart speed: 30 in./hr; detector: flame ionization).

RESULTS

Straight chain hydrocarbons, fatty acids, and fatty alcohols of *C. pluricaulis* (Chois) were separated by column chromatography on alumina using various combinations of petroleum ether (40-60 C), benzene, and chloroform, and compounds were identified by GLC (Table II). *C. pluricaulis* (Chois) was found to contain C_{22}-C_{33} hydrocarbons, C_{27}, C_{31}, and C_{33} as the major components. Fatty acids were mainly of even number carbon atoms ranging from C_{14}-C_{28}. Palmitic, myristic, and linoleic acids were the major components. The wax-alcohol fraction was found to consist of even number carbon atoms ranging from C_{24}-C_{32}. In relative concentration, C_{26}, iso-C_{28}, iso-C_{30}, and C_{32} were found to predominate.

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TABLE II

Distribution of Wax Hydrocarbons, Fatty Acids, and Fatty Alcohols by Gas Liquid Chromatography

Carbon chain length	Percent Composition		
	Hydrocarbon	Acid	Alcohol
14	-	25.3	-
15	-	Traces	-
16	-	58.7	-
17	-	-	-
18	-	Traces	-
18:1	-	Traces	-
18:2	-	3.5	-
20	-	1.2	-
21	-	Traces	-
22	Traces	1.5	-
24	-	Traces	-
24	-	2.0	1.6
25	Traces	Traces	-
Iso-26	-	Traces	2.7
26	Traces	Traces	50.2
27	15.8	-	-
Iso-28	-	-	4.0
28	-	Traces	-
29	1.7	-	-
Iso-30	-	-	15.8
30	Traces	-	-
31	61.2	-	-
Iso-32	-	-	-
32	-	-	3.1
33	10.5	-	-
Iso-34	-	-	-

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[Received December 4, 1974]

The Stereochemistry of Linoleic Acid Tetrabromides

ABSTRACT

The NMR spectrum of the crystalline tetrabromide obtained from bromination of linoleic acid exhibited an AA'XX' splitting pattern for the C-11 hydrogens, indicative of a racemic structure rather than a meso for the brominated part of the molecule. Thus, the crystalline isomer is a racemic mixture of 9 R, 10 R, 12 R, 13 R-tetrabromooctadecanoic acid and 9 S, 10 S, 12 S, 13 S-tetrabromooctadecanoic acid. The C-11 methylene spectrum of the liquid tetrabromide was treated as an ABX_2 pattern; thus, this isomer is a mixture of 9 R, 10 R, 12 S, 13 S-tetrabromooctadecanoic acid and 9 S, 10 S, 12 R, 13 R-tetrabromooctadecanoic acid.

INTRODUCTION

The bromination of linoleic acid produces two tetrabromides, a crystalline isomer and a liquid isomer. The crystalline isomer is an important intermediate in the preparation of pure linoleic acid (1) and in the preparation of labeled linoleic acid (2). *Trans* addition of bromine to a *cis* alkene gives the threo dibromide, hence addition of two moles bromine to *cis, cis*-octadecadienoic acid (linoleic acid) would be expected to give two different threo, threo racemic mixtures (3). These two pairs of enantiomers are diastereomeric and differ in the relationship of the 9, 10 chirality to the 12, 13 chirality (Fig. 1). The enantiomeric 9 S, 10 S, 12 R, 13 R-tetrabromooctadecanoic acid (I) and 9 R, 10 R, 12 S, 13 S-tetrabromooctadecanoic acid (II) have a pseudoplane of symmetry through C-11 (opposite chirality at C-10 and 12), whereas the other enantiomeric pair (III and IV) have a pseudoinversion plane through C-11 (same chirality at C-10 and 12). The question is which pair is the crystalline tetrabromide and which is the liquid.