New, Unusual Long Chain Fatty Acid (Argemonic Acid) from *Argemone Mexicana*

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ABSTRACT

An unusual long chain fatty acid has been isolated from argemone oil. By chemical degradation and by spectral analysis, the acid now is shown to be (+) 6-hydroxy-6-methyl-9-oxo-octacosanoic acid and designated as argemonic acid.

INTRODUCTION

Argemone oil contamination in edible oils is implicated in epidemic dropsy (1). An alkaloid sanguinarine which belongs to the isoquinoline group is present in the oil and is shown to be related etiologically to the toxic manifestations (2,3). A recent report of epidemic dropsy from Andhra Pradesh in India showed a deliberate adulteration of argemone oil in edible oils (4). Analysis of urine and blood samples of these affected patients showed the presence of sanguinarine (5). Studies on animals in this Institute (6) indicated that, in addition to sanguinarine, other factors in the oil also could be responsible for potentiating argemone oil toxicity. This prompted a thorough chemical investigation of the oil. Studies from this Institute (7), as well as elsewhere (8), indicated the presence of an unusual fatty acid in the oil. This paper presents the structure of the unusual fatty acid which is named as argemonic acid (7). However, its specific role in argemone oil toxicity is yet to be elucidated.

RESULTS AND DISCUSSION

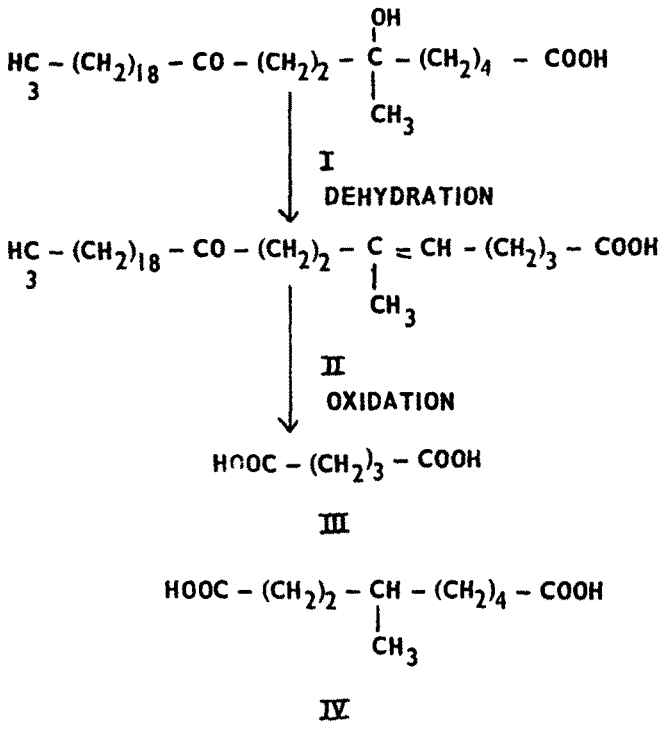
The isolation of an unusual fatty acid from argemone oil already has been described in a previous report (7). It was found to be an aliphatic polar (thin layer chromatography [TLC]) compound melting at 92-93 C with optical activity $(\alpha)_D^{23}$ + 7.2, with a molecular formula $C_{29}H_{56}O_4$ on the basis of elemental analysis and mass spectra (M^+ 468). The acid formed a crystalline methyl ester mp 70-72 C, $C_{30}H_{58}O_4$ (M^+ 482). The IR spectrum of the methyl ester (1710 cm^{-1}), (1738 cm^{-1}) showed the presence of a >C=0 group in the molecule though the acid showed only one peak at 1703 cm^{-1} which may be due to overlapping of the ketone and carboxylic acid carbonyl with the resulting enhancement of intensity. The NMR spectrum of the acid showed signals for protons α- to carbonyl (δ 2.35 ppm) in addition to the shielded methylene signals as a singlet at (δ 1.3 ppm) and also a triplet for terminal methyl at (δ 0.9 ppm). Gas liquid chromatography (GLC) of the silylated methyl ester of the acid showed a single peak at 220 C on a silicone column with temperature programing, suggesting that the compound may be a long chain hydroxy aliphatic acid.

The acid showed no evidence of unsaturation, since it was not affected by catalytic hydrogenation over PtO_2. Acetylation with pyridine-acetic anhydride at 50 C did not take place. However, acetylation with acetic anhydride-perchloric acid at room temperature furnished an acetate, melting at 84-86 C, showing the presence of a hindered hydroxyl.

Lithium aluminum hydride (LAH) reduction (9) gave a crystalline trihydroxy compound ($C_{29}H_{60}O_3$), melting at 89-90 C. It has a strong absorption at 3000 cm^{-1} in IR with the disappearance of the 1710 cm^{-1} peak. The trihydroxy derivative formed a crystalline diacetate (Py/AC_2O, $C_{33}H_{64}O_5$), melting at 80-82 C, and a triacetate (Py/perchloric acid, $C_{35}H_{66}O_6$), melting at 86-87 C.

Evidence of the presence of the carbonyl group in the molecule was established by converting the >C=0 group to an amide by Beckmann rearrangement. Under controlled conditions (10), an amide was obtained as a major product. The amide was hydrolyzed when a mixture of unsaturated dicarboxylic acids and an amine was obtained. The amine was identified as $(CH_3(CH_2)_{18}NH_2$ by comparing with the amine obtained from authentic eicosanoic acid by Hofmann reaction. The amine had the same R_f value on paper chromatography in two solvent systems and a superimposable IR as that of the authentic amine. The unsaturated dicarboxylic acid (NMR δ 5.4 ppm as a triplet for vinylic protons) was hydrogenated with Pd/C and subsequently methylated. The methyl ester had a retention time of 10.3 min at 215 C on GLC with diethyleneglycol succinate (DEGS) column on temperature programing. Authentic sebacic acid was obtained from castor oil on strong alkali fusion. The TLC and IR behavior of the dicarboxylic acid obtained, differed from authentic sebacic acid. in IR, it showed $\sqrt{}_{max}$ 1380 cm^{-1} and two overlapping bands with absorption maxima at 1285 cm^{-1} and 1235 cm^{-1} indicating the branched methyl group near the carboxyl ends (11). In other respects, the IR is identical with authentic sebacic acid, indicating that the dicarboxylic acid is isosebacic acid (IV).

The position of the hydroxyl in argemonic acid methyl ester was fixed by subjecting to dehydration with p-toluenesulphonic acid when a mixture of products was obtained (Scheme 1). On separation over a column of silicic acid, 60% of the product (II) was obtained by eluting with chloroform-methanol (9:3). This compound (homogeneous on TLC) showed the signal at δ 5.4 ppm as a triplet in NMR for vinylic protons. The product (II) was subjected to oxidation with peracetic acid (12) (oxirane ring IR 870 cm^{-1}) and then hydrolyzed to give a mixture of glycols (13). Another part of the dehydrated product (II) was subjected to permanganate-periodate oxidation (14) when a mixture of acids were obtained. On separation over a column of silicic acid using citrate buffer (15), glutaric acid and succinic acid were identified by the superimposable IR and also by identical R_f values on paper chromatography with authentic glutaric acid and succinic acid. A minor quantity of a keto-monocarboxylic acid also was obtained which could not be identified due to low yield. These reactions suggest a partial structure as $CH_3(CH_2)_nCO(CH_2)_{26-n}$-COOH with a hydroxyl on one of the methylene carbon atoms. The major peaks in the mass spectrum are accounted



SCHEME 1. Degradation of argemonic acid.

by this structure for argemonic acid.

The molecular ion peak is at M^+ 468 and the base peak at 215. It is well known that McLafferty rearrangement takes place at >C=0. The major fragments are shown in Table I.

Thus, the >C=0 group is fixed at the ninth carbon atom in the molecule. The formation of glutaric acid (III) suggests the position of the tertiary hydroxyl at C_6, where there is a methyl branching which is substantiated by the formation of isosebacic acid (IV).

Thus, from the foregoing chemical and spectral studies and the optical rotation, argemonic acid is assigned the structure (+) 6-hydroxy-6-methyl-9-oxo-octacosanoic acid (I).

EXPERIMENTAL PROCEDURES

Mp were uncorrected. IR spectra were taken using a Perkin-Elmer 221 spectrophotometer and KBr pellets unless otherwise indicated. NMR was recorded at 60 Hz with trimethylsilyl (TMS) as internal standard and medium in $CDCl_3$ with values expressed as δ units. Mass spectra were taken on a Perkin Elmer model 270 B instrument. GLC was carried out on an F&M model 1609 unit with flame ionization detector. The stainless steel column (2 x 3/16 in.) was packed with 5% SE-30 on Chromosorb W (45-60 mesh). Separations were carried out by chromatographing the silylated esters with temperature programing 200-280 C at 5 C/min.

Argemonic acid was isolated either by adding excess of petroleum ether to the screw pressed oil when it precipitates or by filtering the precipitate which settles down when the petroleum ether extracted oil is kept at room temperature for 25 hr. The crude precipitate was washed with methanol and crystallized from hot methanol as colorless crystals melting at 92-93 C, $(\alpha)_D^{23}$ + 7.2 ($CHCl_3$C, 2.5%), C, 74.20% H, 12.02%. It showed a single spot on TLC, IR (KB4) 1703 cm^{-1}, and no characteristic UV absorption. NMR ($CDCl_3$) δ 2.35, multiplet, 6H (-CO-CH_2) ppm, δ 1.3, singlet, (-CH_2) ppm, δ 0.9, triplet, 3H, (-CH_3) ppm. The branched methyl signals were not clear and may be masked by the long methylene proton signals.

TABLE I

Mass Fragmentation Pattern of Argemonic Acid

	m/e	Percentage
H_3C-$(CH_2)_{18}$	267	28
H_3C-$(CH_2)_{18}$-C(=O)•	295 intense	20
H_3C-$(CH_2)_{18}$ - C(OH)CH_2	310	35
H_3C-$(CH_2)_{15}$ - CH=CH_2	252	12
HO(OH)H_2C - C - $(CH_2)_8$ - COOH	215	100
O=C(OH)-$(H_2C)_8$ - COOH	201	40
$C_{29}H_{56}O_4$	468 molecular ion	M^+ 3
$C_{28}H_{53}O_4$	453	5
$C_{27}H_{50}O_4$	438	5
$C_{28}H_{55}O_2$	424	5

The acid has no double bonds, as it is unaffected by catalytic hydrogenation over PtO_2 in ethanol at 45 C at atmospheric pressure for 2 hr. It has no rings, as there is no indication by any physical or chemical analysis. Molecular formula $C_{29}H_{56}O_4$ was obtained by mass spectra and elemental carbon and hydrogen analysis.

Methylation: The acid (100 mg) was treated with ethereal diazomethane at 0 C for 24 hr. After the reaction, ether was removed and the residue recrystallised from methanol when colorless needles melting at 70-72 C were obtained. IR $\sqrt{}^{KBr}_{max}$ 1705 cm^{-1}; NMR ($CDCl_3$) δ 3.67, s, 3H (-OMe) ppm, δ 2.3, m, 6H (-CO-CH_2) ppm; δ 1.27, s, (-CH_2) ppm, δ 0.9, t, 3H (-CH_3) ppm, $C_{30}H_{58}O_4$ (*m/e* 482, C, 74.36; H, 11.98, $C_{30}H_{58}O_4$ requires (C, 74.68; H, 12.07%). The purity of the ester was confirmed by TLC. The silyl ether on GLC, with temperature programing, gave a single peak at 220 C. The ester is unaffected by treatment with pyridine-acetic anhydride at 50 C. It forms an acetate with pyridine-perchloric acid at room temperature for 24 hr, mp 84-86 C. Found C, 72.94, H, 11.10%; $C_{32}H_{60}O_5$ requires C, 72.23, H, 11.52%.

LAH reduction: Argemonic acid (100 mg) in tetrahydrofuran (200 ml) was stirred with addition of lithium aluminium hydride for 6 hr at 60 C. After the reaction, it was filtered and evaporated, and the hydroxy compound was twice recrystallized from methanol as white crystalline flakes melting at 89-90 C, showing a single spot on TLC (Found C, 76.89; H, 13.4% calculated for $C_{29}H_{60}O_3$; C, 76.32; H, 13.16%); IR $\sqrt{}^{KBr}_{max}$ 3000 cm^{-1} with the disappearance of 1710 cm^{-1} peak. Diacetate (Py/Ac_2O) mp 80-82 C; C, 73.14; H, 11.68%; calculated for $C_{33}H_{64}O_5$: C, 73.33; H, 11.85%. Triacetate (py/perchloric acid) mp 86-87 C; C, 72.54; H, 11.21%, calculated for $C_{35}H_{66}O_6$: C, 72.16; H, 11.34%.

Oxime formation and rearrangement: Argemonic acid methyl ester (150 mg), hydroxylamine hydrochloride (70 mg) in water (5 ml), potassium hydroxide (85 mg), and ethanol (10 ml) were refluxed for 6 hr. After the reaction was over, the contents were evaporated, water was added, and the precipitate filtered. The white crystalline oxime obtained was stirred with concentrated sulphuric acid (4 ml) for 6 hr on a water bath. After the reaction, the mixture was poured into water and filtered. The crude amide obtained was passed over a column of silicic acid. The ether-methanol eluate contained the major 80% of the amide which on crystallization from methanol yielded colorless flakes mp 107-108 C (85 mg).

Hydrolysis of amide: The amide (82 mg) was hydrolyzed

with aq KOH (15 ml; 30%) and autoclaved for 6 hr at 180-200 C. On cooling, the contents were acidified with 15 ml 6 N HCl and steam distilled. The residue was extracted with petroleum ether and then with ether and dried over sodium sulphate. The aqueous layer was passed over a column of Dowex I x 8 (100-200 mesh) (1 x 50 cm) and eluted with water. Fractions 8-10 (50 ml) were basic to litmus and were pooled; the water was evaporated, and acetone was added to precipitate the amine. This afforded a sticky mass which gave a single spot on paper chromatography. Found C, 78.40, H, 16.21%; $C_{19}H_{41}N$ requires C, 78.55, H, 16.72%. Authentic eicosanoic acid was converted into the amine by Hofmann's reaction. The amine obtained had a superimposable IR and identical GLC retention time with the amine obtained from the argemonic acid.

The petroleum ether layer, on evaporation, yielded monocarboxylic acid in very low yield which could not be identified. The ether layer on evaporation gave an unsaturated dicarboxylic acid (20 mg) NMR δ5.4 ppm t, (-CH=CH) ppm for vinylic proton. It was hydrogenated (Pd/C) and subsequently methylated (diazomethane). On GLC on DEGS column, it had a retention time of 10.3 min. The dicarboxylic acid on TLC with solvent system chloroform-methanol (4:1) had an R_f of 0.48, whereas authentic sebacic acid obtained from castor oil had an R_f of 0.56 under identical conditions. IR $\sqrt{}^{KBr}_{max}$ 1380 cm^{-1}, 1230 cm^{-1}. NMR ($CDCl_3$) δ 1.12, (-$\overset{|}{\underset{CH_3}{C}}$-) ppm.

Dehydration: Argemonic acid (50 mg) and p-toluenesulphonic acid (25 mg) were refluxed in ethanol (20 ml) for 6 hr. The ethanol was removed completely, and water (100 ml) was added and extracted with chloroform (20 ml x 3). The chloroform extract was dried over sodium sulphate and evaporated. The product on TLC showed a mixture of three compounds. It was passed over a column of silicic acid (2 x 35 cm), and the major fraction obtained by elution with chloroform-methanol (4:1) was collected and recrystallized from methanol (20 mg). NMR ($CDCl_3$) δ 5.34, t, (-CH=CH) ppm for vinylic protons, δ 2.4, m, 6H (-CO-CH_2) ppm, δ 1.25, s, (-CH_2) ppm, and δ 0.9, t, 3H (-CH_3) ppm.

Oxidation with peracetic acid and hydrolysis: The dehydrated product (30 mg) was subjected to oxidation with peracetic acid (11). After working up, the epoxide (IR 870 cm^{-1}) was hydrolyzed by refluxing with alcoholic 4 N HCl for 4 hr. The alcohol was removed, water was added and extracted with chloroform. The chloroform layer was dried over sodium sulphate and evaporated. The product on TLC showed three spots.

Periodate-Permanganate oxidation (14): The dehydrated product (20 mg) in tertiary butanol (10 ml) was treated with the oxidant solution (5 ml) containing 20.86 g (97.5 mM) sodium metaperiodate and 250 ml 0.01 M (2.5 mM) $KMnO_4$/liter and potassium carbonate (1 g) in water (4 ml). The mixture was kept shaken for 2 hr at room temperature and then sodium bisulphite (5-8 mg) and 2 ml 50% H_2SO_4 were added to stop the reaction. It was extracted with chloroform, and the chloroform extract was dried over sodium sulphate. The chloroform was evaporated and the crude product Methylated (Me-OH-HCl) and passed over silicic acid and eluted with citrate buffer following the procedure of Higuchi, et al. (15). A dicarboxylic acid (5 mg) identical in behavior on TLC and IR with glutaric acid; and another dicarboxylic acid (4 mg) identical in behavior on TLC and IR with succinic acid; and a ketocarboxylic acid (2:4 DNP) in minor quantities were obtained.

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