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# FRUIT TERPENOIDS AND FATTY ACIDS OF STRYCHNOS SPINOSA LAM.

F. N. I. MORAH

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## ABSTRACT

**Chemical investigation of the pericarp of the versatile African medicinal plant, *Strychnos spinosa*, which was aimed at identifying the pharmacologically active component, has lead to isolation of ursolic acid. The work has suggested the possibility of ursolic acid being the active principle against dysentery in this plant. The seed oil was also analysed and was found to consist of α - amyrin, palmitic, stearic, oleic and linoleic acids. All these compounds are being identified for the first time in this plant specie.**


***Key words:*** ***Strychnos spinosa*, α -amyrin, ursolic acid, seed oil, dysentery**

## INTRODUCTION

*Strychnos spinosa* Lam. (Loganiaceae) is a deciduous shrub and native to tropical and Southern Africa. It is reputed to be a versatile medicinal remedy against many diseases (Bisset, 1970). The seeds are said to possess emetic action and are used in some parts of Africa as an antidote against snake bite. The pericarp is also widely used in Mozambique against dysentry. Bisset and Phillipson (1971) suggested the presence of strychnine and icajine alkaloids in *S. spinosa* on the basis of tlc. Leaves and stem extract from Zimbabwe's sample were found to have muscle-relaxant activity and contained eight unidentified alkaloids in trace amounts (Bisset et al, 1971). Oguakwa et al (1980) isolated and identified akagerine, kribine and 10-hydroxy-akagerine alkaloids from the leaves of *S. spinosa*. In a previous paper (Adesogan and Morah, 1981) we reported the isolation of stryspinolactone from the seeds. (+) - lirioresinol B, which has a strong antibiotic activity was isolated from the seeds (Morah, 1982). Other compounds isolated are 12-hydroxy-11-methoxy-diaboline (Ohiri *et al*, 1981), kingside (Msonthi *et al*, 1985), nonacosanol and nonacosane (Morah, 1993). The fruit pulp has also been shown to contain a high concentration of soluble sugars (Farinu, 1986). The present paper reports on ursolic acid and terpenoids of *S. spinosa*.

## EXPERIMENTAL

All melting points were taken on a hot-stage microscope, and are uncorrected. Infrared quoted are for nujol mull and were taken on a Perkin Elmer model 137 instrument. Ultraviolet spectra were recorded in methanol solution, on a Perkin Elmer model 137 UV instrument. NMR spectra were recorded on varian T-60 instrument, in deuterochloroform with TMS as an internal reference. Silica gel refers to MN (Mechery, Nagel and Co) mesh 0.05nm-0.20nm. Light petroleum refers to the fraction bp 60-80.° Mass measurements were recorded using Varian MAT 1125 instrument at 70 e.v., relative intensity values are quoted in parenthesis.

## PLANT MATERIAL:

Strychnos spinosa fruit was collected in January from Borgu Games Reserve, Kwara State. The plant was identified by the Federal Department of Forestry Research, Ibadan, with whom a herbarium specimen NO FH 1 94093 is filed.

## THE SEED EXTRACT:

The dry seeds (4.25kg) were ground, and extracted with light petroleum for 18hr. The petroleum extract was concentrated down to give an oil (83g). This oil was mixed with dry ethanol (300cm$^3$) and KOH (28g) and refluxed vigorously for 3.5 hr. The excess ethanol was distilled off and the residue caked on cooling. The cake was dissolved in hot water, cooled and extracted with ether to give a non- fatty acid portion (12g). The aqueous fraction was acidified with HCl and extracted with ether to give the mixed fatty acids (53g).

The aqueous layer was distilled down until precipitation of KCl set in. The material was mixed with anhydrous sodium sulphate, dried in a desiccator under vacuum for two days and extracted with acetone for 2.5 hr in a Soxhlet extractor to give glycerol (6g), vmax 3289 and 2899 cm$^{-1}$

Chromatography of the ether soluble non-fatty acid fraction over alumina afforded two crystalline solids. The first was eluted by 5 and 10% ether in light petroleum and crystallised as white needles (8g), mp 184 -186°. IR vmax (3280;1377;1355;1099; 1040; 1027 and 995)cm$^{-1}$ MS m/z426 (M$^+$, 46); 218 (100); 207 (36); 203 (10) and 133 (29); $^1$HNMR δ5.12 (1H,t,J= 3Hz); 3.80 (IH,m); 1.16(3H,s); .1.00 (6H,s); 0.95 (3H,s) and 0.79 (12H,s).

F. N. I. MORAH, Department of Chemistry, University of Calabar, Calabar, Nigeria

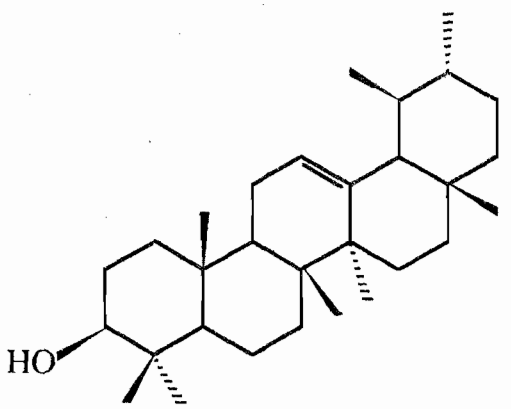


Fig 1: α - amyrin

Acetylation of this triterpene alcohol (100mg) with pyridine and acetic anhydride afforded an acetate mp 224-226°; max 1724 $cm^{-1}$ and MS,m/z 468 ($M^+$) and 218 (100). This triterpene was identified as α- amyrin. (Sprung, *et al.*, 1937).

The second solid, eluted by 50% ether in petrol, was identified as β- sitosterol (1g), mp 136-137°; MS, m/z 414 ($M^+$) Acetate mp 132-133°.

The mixed fatty acid (2.1g) was mixed with dry MeOH (20$cm^3$) and refluxed witih dry HCl gas for 4.5 hr. The excess MeOH was distilled off and the residue taken into light petroleum, washed with aqueous sodium hydrogen carbonate, dried over anhydrous sodium sulphate and distilled to give the methylester as an oil (2.3g). Glc analysis of the oil was taken and calibrated with known samples,. Four peaks corresponding to palmitic, oleic, stearic and linoleic acid methylesters were observed.

## PERICARP EXTRACT:

The pericarp of *S. spinoa* fruits (8kg) was crushed, defatted with light petroleum for 26 hr. and extracted with methanol for 26 hr. in a Soxhlet extractor. The methanol extract was partitioned between water and chloroform.

The $CHCl_3$ extract was chromatographed over silica gel. 50% ether in ethyl acetate eluted a solid which crystallised from MeOH/acetone, mp 284-286°. IR vmax {3367 (OH), 3300-2618 (broad, OH), 1678 (C=O), 1287, 1266, 1248, 1231, 1179, 1024 and 922} $cm^{-1}$. $^1H$ NMR δ 5.15 (IH,bs), 4.48 (IH,t,J= 7.4 (Hz), 2.01 (3H,s, - $OCOCH_3$), 1.60 (3H,s), 0.83 (9H,s) and 0.76 (3H,s).

The hydroxy acid (250mg) was suspended in dry MeOH (25 $cm^3$). Iodomethane (0.5g) and anhydrous $K_2CO_3$ (0.5g) were added and stirred for 32hr. The reaction, on work up, gave the methylester as white plates (230mg) from MeOH, mp 171.5-173° IR vmax {3597 (OH) and 1717 (C=O)} $cm^{-1}$; 1H NMR δ 3.18 (1Ht,J =3Hz), 3.57 (3H,s, $CO_2CH_3$) and 2.95-3.35 (1H.bs). Acetylation of the methyl ester afforded the methyl ester acetate as white needles from MeOH, mp 242-244°, IR vmax 1724 $cm^{-1}$ 1H NMR δ 5.18 (1H,t, J= 3Hz) 4.45 (1Hz) 3.57 (3H,s) $CO_2CH_3$) and 2.10 (3H,s,0. $CO.CH_3$ ), 1.07 (3H,s Me), 0.93 (6H,s, 2 Me), 0.85 (9H,s, 3Me), 0.75(3H,s Me) and 2.00-1.00 (m. methylenes) The hydroxy acid was identified as ursolic acid.

## RESULTS AND DISCUSSION

*Strychnos spinosa* seed oil on ethanolic KOH saponification gave the ether soluble non-fatty acids. Chromatography of the ether soluble non-fatty acid portion on alumina afforded two solids. 5% and 10% ether in light petroleum eluted a white solid mp 184-186° (acetate, 224-226° [1] identified as α -amyrin (fig.1) by comparison of its mp,. ir and NMR with literature data (Shama et al., 1962; Ogukoya., 1981; Sprung et al, 1937). The second solid eluted by 50% ether in light petroleum was identified as β- sitosterol

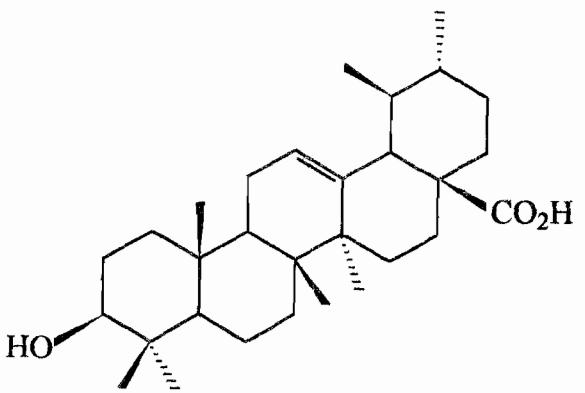


Fig 2: ursolic acid

by comparison of its MP, IR, NMR with literature data (Friedland, 1959).

Gas chromatographic analysis of the methyl ester of the mixed fatty acid revealed it to be a mixture of four fatty acid esters identified as methyl palmitate (14.1%), methyl stearate (23.8%), methyl oleate (6.10%) and methyl linoleate (55.8%). The different peaks on the gc chart were identified by calibration with known standards. The peaks were measured by triangulation and the calculated composition converted from weight to mole percent.

The chloroform fraction of the methanol extract of petrol defatted *S. spinosa* pericarp on chromatography over silical gel afforded a triterpene hydroxy acid identified as ursolic acid (fig. 2) by comparison of its ir,mp, NMR with literature data (Shama et al, 1962) The ursolic acid is biogenetically related to α- amyrin, isolated from the seed, from which it can be obtained by hydroxylation of the $C_{-28}$ methyl followed by oxidation to form the acid group.

The occurrence of ursolic acid as the major secondary metabolite of the *Strychnos spinosa* pericap is of interest because the fruit pericap is widely used in Mozambique against dysentery and the same ursolic acid is a major metabolite of *Stachytarpheta indica* also used against dysentery (Ngir et al, 1974). Ursolic acid is also known to have anti-inflammatory properties as well as a chain of other physiological activities (Shitilo, 1973). It is therefore being inferred here that ursolic acid is a

possible principle of this species against dysentery and some other human diseases. It may then be considered along with (+) - lirioresinol B (Morah, 1982) which has antibiotic properties as the pharmacologically active components of this versatile African medicinal plant.

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