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ISOLATION AND STRUCTURAL DETERMINATION OF COMPOUND AM-2 FROM THE STEMBARK OF *ANNONA MURICATA LINN*

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ABSTRACT

Annona muricata Linn, belongs to the family Annonaceae. Oil from seeds of some plants may be used for the production of edible oils and soaps. Finally, many members of this family are used in folk medicines for various purposes. Annona muricata roots are used as anti-spasmodic, parasiticidal. Flower and flower buds as a bechic. The unripe fruits is useful as anti-scorbutic. Seeda are useful as fish-poison, insecticidal and astringent. The compounds that are isolated from A. muricata are Anomuricine, Anomurine, Coclaurine, Reticuline, Coreximine, Stepharine, Atherosperminine, Anonaine and Liriodenine. Compound AM-2 was identified as Murisolin. AM-2 was isolated as waxy material, which melted just above room temperature 30^0 C. The molecular formula of AM-2 was settled as $C_{35}H_{64}O_6$ on the basis of the results of elemental analysis.

KEY WORDS: *Annona muricata*, Squamosten, Squamocin, Murisolin, Annonaceae.

INTRODUCTION

*Annona muricata Linn***,** belongs to the family Annonaceae which is a large family of tropical and subtropical trees and shrubs comprising about 120 genera and more than 2000 species. Economically the family is of appreciable importance as the source of edible fruits, the pawpaw (*Asimina*), cherimoya, custard apples, sweet sop, sour sop and ilama (*Annona*) and fruits of the genera *Canaga* and *Rollinia.* Oil from seeds of some plants may be used for the production of edible oils and soaps. Finally, many members of this family are used in folk medicines for various purposes.

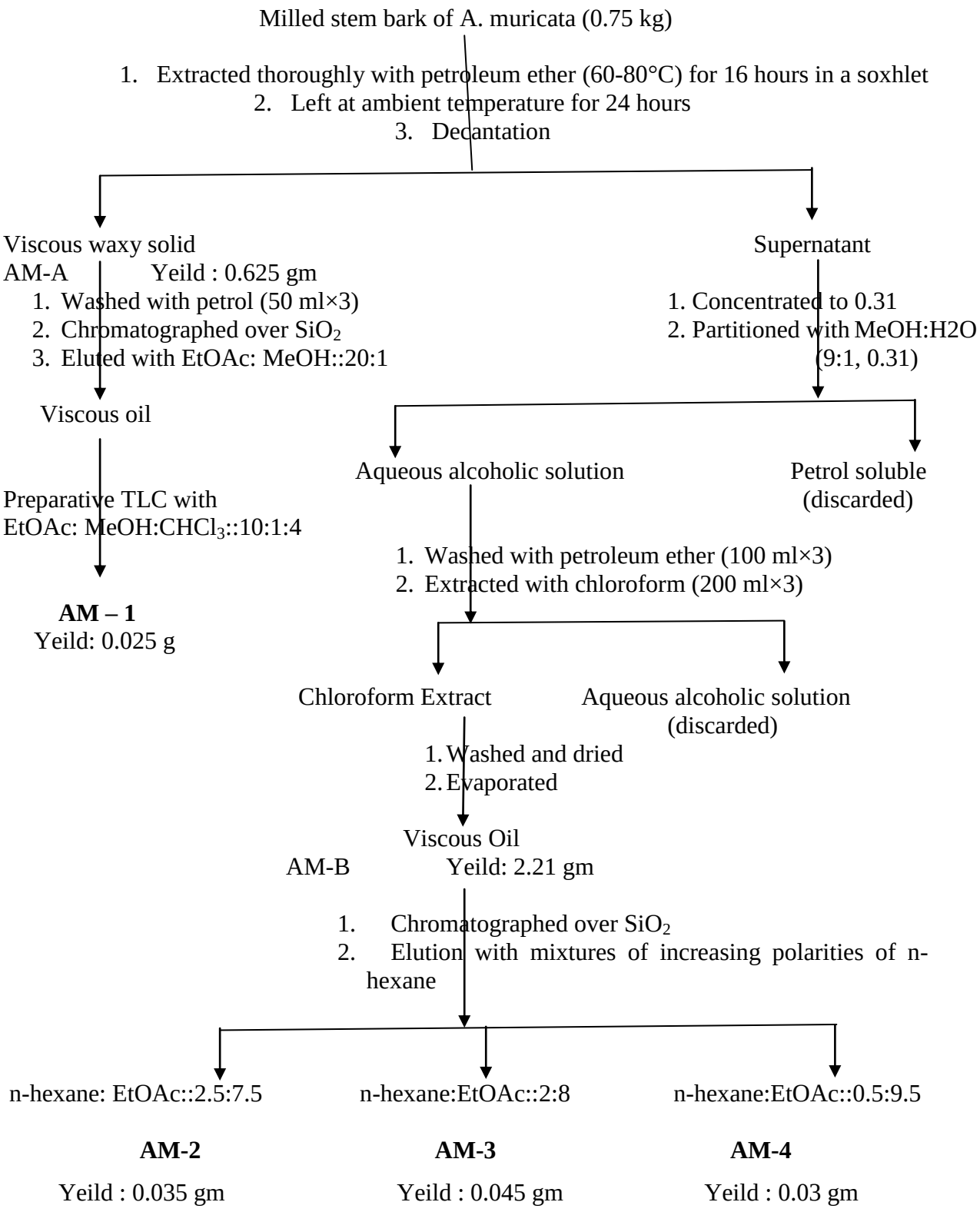
Morphological Descriptions: It is a small being glabrous when old. The leaves are large, ovate, obovate, acute or bluntly acuminate, rounded at the base, glabrous; blade 7-2.5 inch, thinly coriaceous, pellucid, punctate, lateral nerves about 12 pairs, prominently seen beneath; flowers in axillary; leaf opposed, pedicelled, few flowered racemes; sepals: triangular, shortly acuminate, pubescent; petals; greenish yellow, usually 3 in numbers, fleshy, triangular, united, thickened and saccate at the base, pubescent on both surfaces about 1 inch long. Pedicles stout; bracteates in the middle, thickened at the tip, one to 2 inch long; fruits: large, globouse, often of irregular growth; carpels do not separate (As in *Asquamosa*), each with an acute tip, giving the surface of the fruit a muricate appearance.

Geographical distribution: *A. muricata* is a native of West Indies. The tree occurs wild and is also cultivated in Cuba, St. bomingo, Jamaica, in gardens near Pune and Mumbai, in Assam and in South India. Apart from *A. muricata*, the following species of *Annona* are also reported to be available in India. *A. squamosal, A. reticulate, A. glabra, A. cherimolia, A. perpurea, A. montana, A. senegalensis and A. atemoya*.

Medicinal Uses: *A. muricata* finds a variety of medicinal uses in traditional system of medicine. The roots are used as anti-spasmodic, parasiticidal. Flower and flower buds as a bechic. The unripe fruits is useful as anti-scorbutic. Seeda are useful as fish-poison, insecticidal and astringent.

Chemistry of *A. muricata:* From the approximately 120 genera and more than 2000 species that are generally considered to make-up the Annonaceae, less than 50 genera and 200 species appear in the chemical literature at all. Even many of the phyto chemical studies of these family reported so far are at best fragmentary. Hance phyto chemical studies and to a lesser extent pharmacological studies on Annonaceous plants have been intensified in the last decade. Most investigations have centered upon allakaloids but Annonaceae also produce a wide range of compounds belonging to various phyto chemical groups. The review paper by Leboeuf et. al. covers the phyto chemistry of Annonaceae up to 1982 which include various allakaloids, carbohydrates, lipids, amino acids, proteins, poly phenols, essential oils, terpenes and aromatic compounds typically found in these plants. Apart from these components, different species of Annona have revealed the presence of a noval group of compounds named Annonaceous acetogenins. The compounds that are isolated from *A. muricata* are Anomuricine, Anomurine, Coclaurine, Reticuline, Coreximine, Stepharine, Atherosperminine, Anonaine and Liriodenine.

PROCEDURE FOR ISOLATION OF CHEMICAL CONSTITUENTS FROM THE STEMBARK OF *A. muricata*



STRUCTURAL DETERMINATION OF COMPOUND AM-2 FROM THE STEM BARK OF *A. muricata*

Compound AM-2 was obtained as a waxy material melted at ≈ 30^0 C. It was found to be freely soluble in Dichloromethane, Chloroform, Ethylacetate and Methanol while sparingly soluble in benzene and practically insoluble in petroleum ether. It was found to be homogenous by HPLC and TLC studies. The TLC plates developed in different solvents systems, when exposed to iodine vapours or spraying with 5% ethanolic sulphuric acid followed by heating the plates at 110^0 C for 10 minutes, a single chromatogram was developed on the plates.

The molecular formula of AM-2 was settled as $C_{35}H_{64}O_6$ on the basis of the results of elemental analysis. This was further corroborated with the results of FAB-mass spectrometrically derived MH^+ ion peak at m/z 581 which established the molecular weight as 580. No satisfactory molecular ion peak was discerned in the EI-MS of AM-2.

The UV spectrum of AM-2 showed absorption maximum at 218nm suggestive of the presence of α,β- unsaturated carbonyl chromophore. The presence of which has further confirmed by its positive test towards Kedde' reagent. The IR spectrum of compound AM-2 showed absorption bands at 1745 cm^{-1}(α,β - unsaturated γ- lactone syatm) 3460, 3590 cm^{-1} (alcoholic hydroxyl group).

The 500 MHz ^{1}H-NMR spectrum of AM-2 was very much similar to several other mono-THF γ- lactone containing acetogenins as well as AM-1. The spectrum showed signals for an olefinic hydrogen, six low field methine hydrogens bond to oxygen functions, forty protons of twenty methylenes and a terminal methyl group.

A careful analysis of ^{1}H-NMR spectrum of AM-2 revealed the presence of subunit in the molecule. The spectral signals at δ 1.43;3H,d(J= 6.8Hz) for te secondary methyl bound to carbon bearing oxygen functions; an Abx system, the AB part of which was centred around δ 2.38, 1H dd (J=13.6 and 7.7 Hz) and 2.52, 1Hdd (J=13.6 and 3.9Hz) with the signals for the x part of ABx system at δ 3.78, 1H, m (overlapping with signal entered around δ 3.80). The signals at δ 2.38 and 2.52 were assigned for the two non equivalent protons on the carbon situated between γ- lactone and a methine hydrogen bearing a hydroxyl group. The

spectrum also showed a quartet of quartets at δ 5.05, 1H, (J=6.8 and 1.4Hz) for the lactonic proton and a quartet at δ 7.20, 1H (J=1.4Hz) for β-proton of α,β- unsaturated γ- lactone.

The ^{1}H-NMR spectrum of AM-2 has also showed additional signals for oxymethine protons at δ3.41, 2H, m and 3.79-3.83, 2H, m. Thus accounted for altogether four additional oxymethine hydrogens, which can best be accounted for the subunit B if AM-2. The hydrogen for methylenes for THF ring were also discerned in the spectrum at δ 1.65 and 2.00, 2H each, m. The presence of subunit C was evidenced by the hydrogen signals at δ 0.87, 3H each, t. (J= 7.4Hz) and δ 1.50, 2H, quintet, thereby accounting for all the six oxygen atoms present in AM-2, three each in subunits A & B.

In order to secure additional information from the NMR spectrum, ^{13}C-NMR spectrum of compound AM-2 was measured at 125 Mhz ($CDCl_3$). The spectrum is consistent with the molecular formula of the compound and accounted for all the 35 carbon present in the molecule. The two methyl carbons gave signals at δc 14.0 and 19.1. The two sp^2 hybridised olifinic carbons of the lactone gave signals at δ 131.1 and 151.7. The spectrum also showed signals for oxycarbons at δc 69.5, 74.0 (2X-CHO-), 78.0 and 82.6 (WX-CHO-). The two low field signals at δc 82.6 (appeared as one signal) was assigned to the oxymethine carbons of the THF ring, while the signals at δc 74.0 (two carbons as one signal) were obviously due to the hydroxyl bearing carbons situated on both the sides of the THF ring. The most upfield signals at δc 69.5 may easily be accounted for the carbon bearing hydroxyl function at C-4 in the subunit A. The oxymethine carbon signal at δc 78.5 and carbonyl carbon signal at δc 173.6 were characteristically due to γ- lactone part of the molecule in subunit A.

On the basis of data presented so far, the subunit B in compound AM-2 must be logically placed some where on the linear acetogenin in such a way that subunits A and C are its two ends, a fact that was revealed on the basis of careful study of mass spectrum fragmentation pattern of AM-2.

The FAB-Mass spectrum of AM-2 showed MH^+ ion peak at m/z 581 whereas the other important fragment ions disconcerted in the spectrum at m/z 563, 545 and 527 were due to sequential loss of three molecules of water from the MH^+ ion.

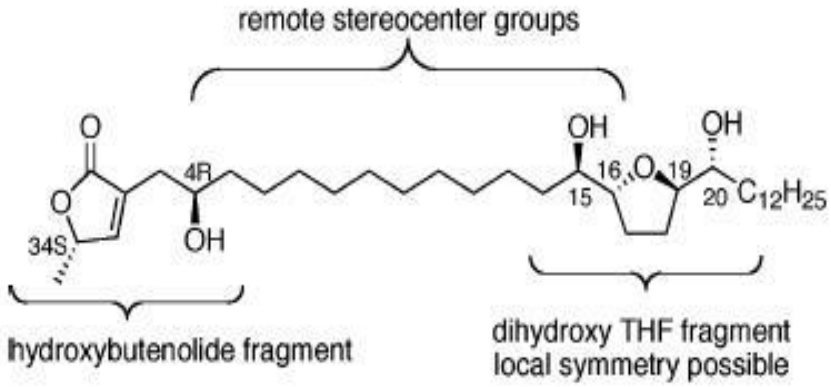
The EI-Mass spectrum of compound AM-2 did not show the molecular ion peak but it was very much informative. The important fragment ions discerned in the spectrum were at m/z 381, 363, 311, 293.

The fragment ions at m/z 141, followed by loss of one molecule of water can be rationalised to the presence of subunit A. The base peak at m/z 311 can only be attributed to the cleavage of C-15/C-16 bond which losses a molecule of water to give an additional peak at m/z 393. The position of THF ring was fixed between C-15/C-20 by the appearance of ion peak at m/z 269 and by formation of fragment ion at m/z 381 formed by cleavage of C-19/C-20 bond. The cleavage of C-15/C-16 bond generated two fragment ions m/z 311 and 269 which accounted for the entire structural representation of the molecule. The similar conclusion was drawn by the formation of two fragment ions at m/z 381 and 199, resulted by the cleavage of C-19/C-20 bond. The structure of AM-2 was thus settled which also gives the genesis of different ions discerned under E1-Mass spectrum of the molecule.

This compound appears to be Murisolin has been earlier isolated from A. muricata seeds. This appears to be isolated from stembark for the first time.

RESULT AND DISCUSSION

On the basis of the data discussed so far the following structure of AM-2 can be determined –



1.14, murisolin
(15*R*,16*R*,19*R*,20*R*), *threo-trans-threo*

The molecular formula of AM-2 was settled as $C_{35}H_{64}O_6$ on the basis of the results of elemental analysis. This was further corroborated with the results of FAB-mass spectrometrically derived MH^+ ion peak at m/z 581 which established the molecular weight as 580.

CONCLUSION

Systemic fractionation of the petroleum ether extract of the bark of A. muricata led to the isolation of 4 compounds which were previously levelled as AM-1, AM-2, AM-3 and AM-4. AM-4 belonged to non-adjacent bis – tetrahydrofuranic acetogenin. Compound AM-2 was identified as Murisolin. AM-2 was isolated as waxy material, which melted just above room temperature 30^0 C. The molecular formula of AM-2 was settled as $C_{35}H_{64}O_6$ on the basis of the results of elemental analysis.

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