WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 8, 1609-1617. Research Article ISSN 2277– 7105

ISOLATION AND STRUCTURAL DETERMINATION OF COMPOUND AM-3 FROM THE STEMBARK OF ANNONA MURICATA

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Article Received on
25 May 2015,

Revised on 17 June 2015,
Accepted on 10 July 2015

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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. On the basis of IR, UV ^{1}H and ^{13}C NMR data, we can decide the Compound AM-3 possess three subunits. The placement of keto-carbonyl group and the THF core on the linear backbone was settled on the basis of analysis of different fragment ions discerned in the E1-MS. The molecular formula of AM-3 was settled as $C_{35}H_{62}O_7$ on the basis of the results of elemental analysis.

KEYWORDS: *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.

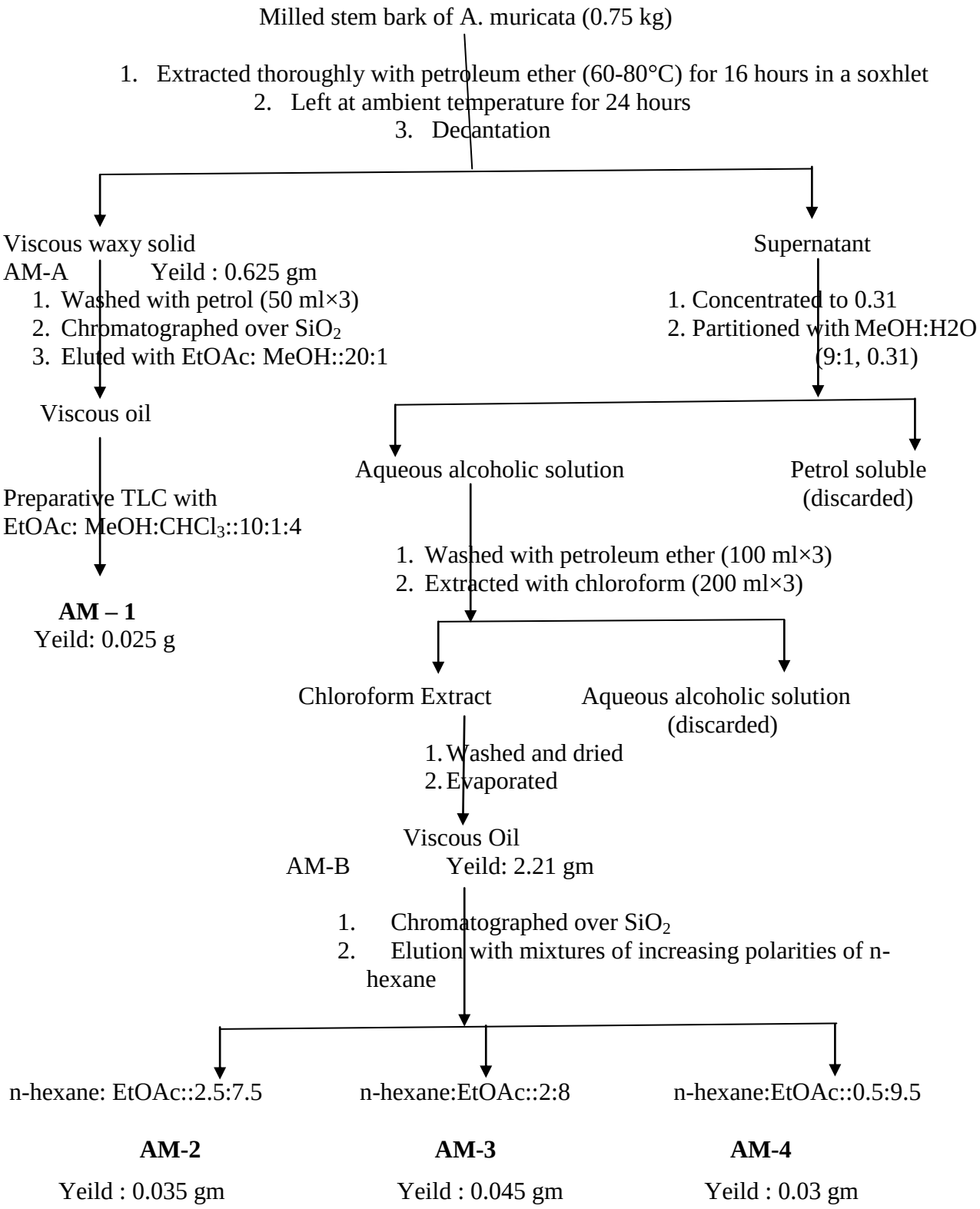
Morphological Descriptions: It is a small being glabrous when old. The leaves are large, ovate, obovate, acute or bluntly acuminate, rounded at the base, glabrous; blade 7-2.5 inch, thinly coriaceous, pellucid, punctate, lateral nerves about 12 pairs, prominently seen beneath; flowers in axillary; leaf 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-3 FROM THE STEM BARK OF *A. muricata*

Compound AM-3 was found to be freely soluble in dichloromethane, acetone, chloroform, ethyl acetate and methanol, while sparingly soluble in benzene and insoluble in n-hexane. It was found to be homogeneous by HPLC & TLC. The TLC plates developed in different solvent systems, when exposed to iodine vapours or spraying with 5% ethanolic sulphuric acid followed by heating the plates at 110°C for 10 minutes or spraying the plates with Dragondorff s reagent, a single chromatogram was detected in the plate.

The molecular formula of the compound AM-3 was settled as $C3_5H_{62}0_7$ on the results of elemental analysis. This was further corroborated with the results of FAB-mass spectrometrically derived MH^+ peak at m/z 595, which established the molecular weight of compound AM-3 as 594. The El-Mass spectrum of compound AM-3 did not give satisfactory molecular ion peak. A comparison of the molecular formula of compound AM-3 with that of compound AM-2 revealed that they are very closely related, with the only difference of the former having two hydrogens less and one oxygen more. This might be due to presence of an additional ketonic group in the molecule.

The UV spectrum of compound AM-3 showed absorption maximum at 225 nm suggesting the presence of an α,β unsaturated carbonyl chromophore in the molecule. The IR spectrum of compound AM-3 (CHCI3) showed absorption bands at 1745 cm^{-1}(α,β -unsaturated γ-lactone system) and 3460, 3590 cm^{-1} (alcoholic hydroxyl group). The positive reaction of the compound towards Kedde's reagent (visualized as a pink spot on the TLC plates upon spraying the reagent) has further confirmed the presence of α,β -unsaturated γ-lactone system in the molecule. The additional IR absorption band at 1710 cm^{-1} indicated a saturated kctocarbonyl moiety in the compound. This IR absorption is in full agreement with its molecular ion peak, which is 14 mass units more in comparison to that of the compound AM-2.

The 500 MHz ^{1}H-NMR spectrum of compound AM-3 ($CDCl_3$) was very much similar to other mono-THF γ -lactone containing acetogenins with a carbonyl function in the linear chain as reported from different Annonaceous plants. It showed the signals for an olefinic proton, six low field methine protons bound to oxygen functions, four protons for two methylenes of mono-THF ring, a secondary methyl group of the carbon bearing oxygen

function, thirty four protons of seventeen methylenes, a terminal methyl group and a four proton signal for two secondary methylenes bound to a carbon bearing oxygen function.

A critical study of the ^{1}H-NMR spectrum of compound AM-3 clearly revealed the presence of subunit A in the molecule. The spectral signals at δ 1.43, 3H, d (J = 6.8 Hz) for the secondary methyl bound to a carbon bearing oxygen function; an Abx system centred at δ 2.38, 1H, dd (J=13.8 & 8.0 Hz) and 2.52, 1H, dd (J=13.8 & 4 Hz) with the signal for the x proton at δ 3.75, 1H, m (partly overlapping with the signal at δ 3.80). The signals at δ 2.38 and 2.52 were for the two non-equivalent protons on the carbon situated between y-lactone ring and a methine carbon bearing a hydroxyl group. The spectrum also showed a quartet of quartets at δ 5.08, 1H (J=6.8 & 1.4 Hz) for lactonic proton and a quartet at δ 7.20, 1H (J=1.4Hz) for the β-proton of the α,β-unsaturated γ-lactone.

The ^{1}H-NMR spectrum of compound AM-3 has shown, in addition to the above signals, the signals for oxymethine protons at δ 3.40, 2H, m and 3.80, 2H, m for altogether four more oxymethine hydrogens. These signals can best be accounted for the subunit B of the compound AM-3. The hydrogens of methylenes of THF ring were also discernible at δ 1.60 and 1.98, 2H each, m. The presence of subunit C was evidenced by the hydrogen signals at δ 0.87, 3H, t and 1.50, 2H, m.

The ^{1}H-NMR spectrum of compound AM-3 showed a four multiplet at δ2.40 (partly overlapping over the signal at δ 2.38), probably due to two methylenes situated on the either side of the carbonyl group (vide supra) in the molecule.

Out of the seven oxygen atoms present in the molecule, six have been accounted in subunit A and B. The seventh oxygen has also been accounted as a part of keto group which may probably be present on the linear side chain of acetogenin either between the THF and the lactone rings or between the THF ring and the tailing end.

In order to secure the additional information from the NMR spectrum, the ^{13}C-NMR spectrum of the compound AM-3 was measured at 125 MHz ($CDC1_3$). The spectrum is consistent with the molecular formula and accounted for all the 35 carbons present in the molecule. The two methyl carbons gave signals at δc 14.0 and 19.0. The two sp^2 hybridised olefinic carbons of the lactone gave signals at δc 130.9 and 151.9. The ^{,13}C-NMR spectrum showed signals for oxygen bearing carbon at δc 69.5, 76.0 (X -CHO-), 82.6 (2X-CHO-) and

78.0. The two low field oxymethine carbons at δc 82.6 (appeared as one signal) were obviously due to the oxymethine carbons of the THF rings, while the signal at δc 74.0 (two carbons as one signal) was assigned to the hydroxyl bearing carbons situated on both the sides of the THF ring. The most upfield signal at δc 69.5 may easily be accounted for the carbon bearing hydroxyl function in the subunit **A.** The oxymethine carbon signal at δc 78.0 and carbonyl signal at δc 174.6 were obviously due to y-lactone ring. The two diagnostic signals at δc 211.4 and 42.5 (2X-C-C=0) clearly indicate the presence of carbonyl carbon on the linear chain of the compound AM-3.

On the basis of the data presented so for, the subunit B in the compound AM-3 appears to be placed on the linear part of acetogenin in such a way that the other subunit A and C are at its two ends, a fact that was verified on the basis of studying its mass fragmentation pattern.

The FAB-Mass spectrum of compound AM-3 gave its MH^+ ion peak at m/z 595. The other important fragment ions discerned in the spectrum at m/z 577, 559, 541 and 523, were due to sequential loss of four molecules of water from the MH^+ ion. The El-Mass spectrum of compound AM-3 did not show any molecular ion peak but it was much informative. The important fragment ions discerned in the spectrum were at m/z 377, 359, 325, 307, 289, 271,269,225,207,197,189,179,141 and 123.

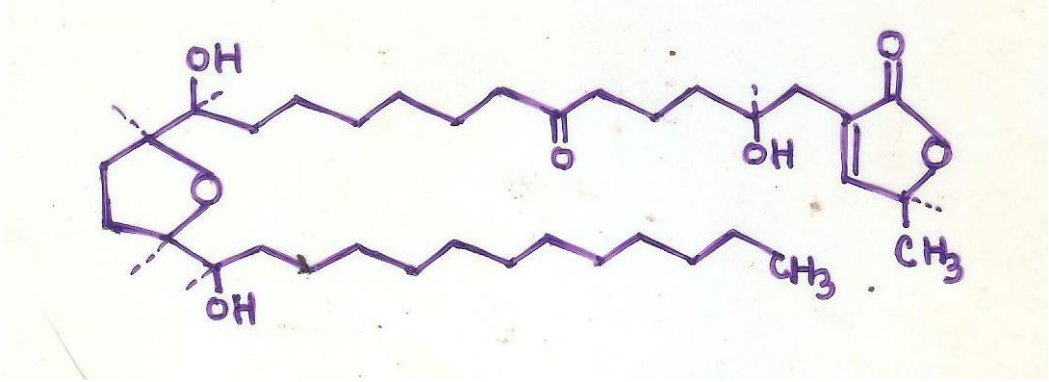
The fragment ion at m/z 225, followed by sequential loss of two molecules of water (ion peaks at m/z 207 & 189) can only be rationalised by the placement of a carbonyl group at C-8. That the subunit A is present in the molecule is evidenced by the signals at m/z 141 and 123 (I4I-H2O). The placement of subunit B between C-14 and C-21 was also proved by the mass peak at m/z 325 (100%), the genesis of which can only be attributed to the cleavage of C-15/C-16 bond.

Three additional oxygen functionalities between C-15 and C-l was evidenced by the ion peak at m/z 307 (325-H_20), 289(325-$2H_20$) and 271(325-$3H_20$). The position of THF ring was further supported by the ion-peak at m/z 269 (formed by the cleavage of C-15/C-16 bond). Thus, the cleavage at C-l 5-C-16 bond generates the two fragments m/z 325 and 269 which accounted for the entire structural representation of the molecule. The scheme shows the mass spectral fragmentation pattern of compound (Structure III) under electron.

Compound AM-3 is a new acetogenin hitherto not reported in literature so far.

RESULT AND DISCUSSION

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



The molecular formula of the compound AM-3 was settled as $C3_5H_{62}0_7$ on the results of elemental analysis. This was further corroborated with the results of FAB-mass spectrometrically derived MH^+ peak at m/z 595, which established the molecular weight of compound AM-3 as 594.

The gross structures of the two isolated mono-THF containing acetogenins were settled as depicted in Schemes 3 and 4, on the basis of spectral data of these compounds. The only problem remained was to settle the stereochemistry of the chiral centres around THF ring and the lactone ring.

The relative stereochemistry of acetogenins AM-2 and AM-3 around the THF core was ascertained by a comparison of the ^{1}H-NMR and ^{13}C-NMR data of these compounds with those of model compound (IV to VII) as developed by Fujimoto et al.

A comparison of the H*NMR spectra of acetogenin AM-2 and AM-3 with those of model compounds in respect of their chiral centres around THF ring shows clearly that the splitting pattern of the isolated acetogenins in the region between 5 3.0 and 4.0 very similar to those of the symmetrical model compound (IV & V) and different from unsymmetrical compounds VI and VII**.** The compounds IV and V are highly symmetrical compounds and they showed just two signals in the region from 8 3.0 to 4.0 ppm, while the compounds VI and VII displayed three signals in the same region. The isolated acetogenins AM-2 and AM-3 have also shown just two signals in this region and therefore they too are also symmetrical possessing either threo-trans-threo or threo-cis-threo configuration around the THF core. Whether it is the former or latter was ascertained with the help of ^{13}C-NMR spectral comparison of carbon resonances of the isolated acetogenins and model compounds.

A careful examination of comparative ^{13}C-NMR spectral data, tabulated for the acetogenins AM-2 and AM-3 and the model compound IV and V for the relevant carbon resonances clearly shows that the two acetogenins possess threo-trans-threo configuration and not threo-cis-tlireo configuration around the THF core, because both the methylenes of THF ring were resonating around δ 28.7 or slightly upfield positions while in the threo-cis-threo configuration, these methylene carbons resonated in the downfield positions. Therefore Schemes 3 and 4, the stereochemical configuration around the THF core is threo-trans-threo.

γ -lactone ring & C-4

The stereochemistry at the chiral centre of γ -lactonc ring of AM-2 and AM-3 was established as S by comparison of CD curve of these compounds together with the CD curve of known compound squamocin where it was settled with degradation method. The chemical shift and splitting pattern of the carbinyl hydrogen at C-4 appeared at similar position in natural product as well as R-(+)Oa-(trifluoromethyl)-Phenylacyl ester derivative when compared with several other known C-4-R acetogenins and corresponding ester, therefore, the chiral centre at C-4 was also considered R like often known annonaceous acetogenins of similar type.

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. On the basis of IR, UV ^{1}H and ^{13}C NMR data, we can decide the Compound AM-3 possess three subunits. The placement of keto-carbonyl group and the THF core on the linear backbone was settled on the basis of analysis of different fragment ions discerned in the E1-MS. The molecular formula of AM-3 was settled as $C_{35}H_{62}O_7$ on the basis of the results of elemental analysis.

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