

Proof-of-presence of unusually naturally occurring homologous series of fifteen saturated odd and even fatty acids in *Acanthus ilicifolius*[#] L. (Acanthaceae)

Shyamaprosad Goswami*, Baidyanath Chatterjee and Mrinal Mallik

B. E. College (Deemed University), Botanic Garden, Howrah-711 103, India

E-mail : spgoswamical@yahoo.com

Fax : 91-33-2668-4564/2916

Manuscript received 22 May 2003, revised 14 November 2003, accepted 14 November 2003

A unique case of unusual presence of the homologous series of fifteen natural saturated fatty acids with molecular formulae $C_{11}H_{23}CO_2H$ to $C_{25}H_{51}CO_2H$ containing all the odd and even members simultaneously has been reported from an Indian medicinal plant, *Acanthus ilicifolius* L. (Acanthaceae). The GC mass analyses of the methyl esters of the mixture of these fifteen acids have been made and their individual mass spectrum thus obtained has been shown as the proof-of-presence of the complete series of the saturated fatty acids in the plant. This unusual case is significant from the biogenetic point of view as the natural presence of such a complete series having even and odd fatty acids simultaneously are unprecedented from one plant source.

A systematic chemical analysis of the medicinal plant, *Acanthus ilicifolius* Linn.¹ (family : Acanthaceae), available in India, Ceylon-Malaya, Philippines, Australia, tropical and S. Africa was undertaken for the active principles for its well known medicinal use specially in the rural Bengal, Konkan, Goa and other parts of India. In the Konkan (India), a decoction of the plant with sugar candy and cumin is given in dyspepsia with acid eructations. In Goa, the leaves are an emollient fomentation in rheumatism and neuralgia. The tender shoots and leaves ground small and soaked in water are applied to snake-bite. In Siam and Cochin China, the plant is considered a cordial and attenuate, useful in paralysis. We have also seen the promising anticancer activity of its crude aqueous extract².

Carboxylic acids constitute one of the most frequently encountered classes of organic compounds and a significant number of natural products. Different natural and synthetic drugs like analgesics, prostaglandins, antibiotics etc. contain carboxylic acid functionality for manifestation of their biological activities. We are interested in the natural mono and dicarboxylic acids as a part of our interest in the possible selective molecular recognition studies with carboxylic acid³ from a mixture of their higher and lower analogues. Fatty acids² are known to be metabolically the most active of plasma lipids and are significant as major building blocks of phospholipids and glycolipids which are important components of biological membranes and as lipophilic modifiers of proteins to tar-

get them to membrane locations. Structural activity studies reveal that long chain saturated and unsaturated fatty acids are more active than the lower members as antibacterials employed mainly as tropical fungicides.

We report herein a novel case of the natural presence of the complete series of fifteen saturated fatty acids having molecular formulae $C_{11}H_{23}CO_2H$ to $C_{25}H_{51}CO_2H$ in this plant from the hexane extract of its stem. Interestingly it was known for a long time⁴ that the acids of natural fats and waxes are characterized by a normal chain with an even number of carbon atoms. C_{16} (palmitic) and C_{18} (stearic) saturated acids are the most common. However some odd carbon fatty acids occur in wool fat. To our knowledge, the presence of such a complete homologous series of fifteen saturated fatty acids has not been previously reported from only one plant source.

Results and discussion

The gas chromatography analysis^{5,6} of the methylated esters of the mixture of these fatty acids is shown as the proof-of-presence of the series of fifteen fatty acids. Interestingly, we have not found the presence of any unsaturated fatty acid in the mixture as is also suggested from the NMR spectral study of the methyl ester derivatives of the mixture of fatty acids.

The fatty acid fraction was isolated from the hexane extract of the powdered stem. Column chromatography over silica gel using petroleum ether and petroleum ether-

*Note the spelling as *Acanthus ilicifolius* in Reference 1.

Note

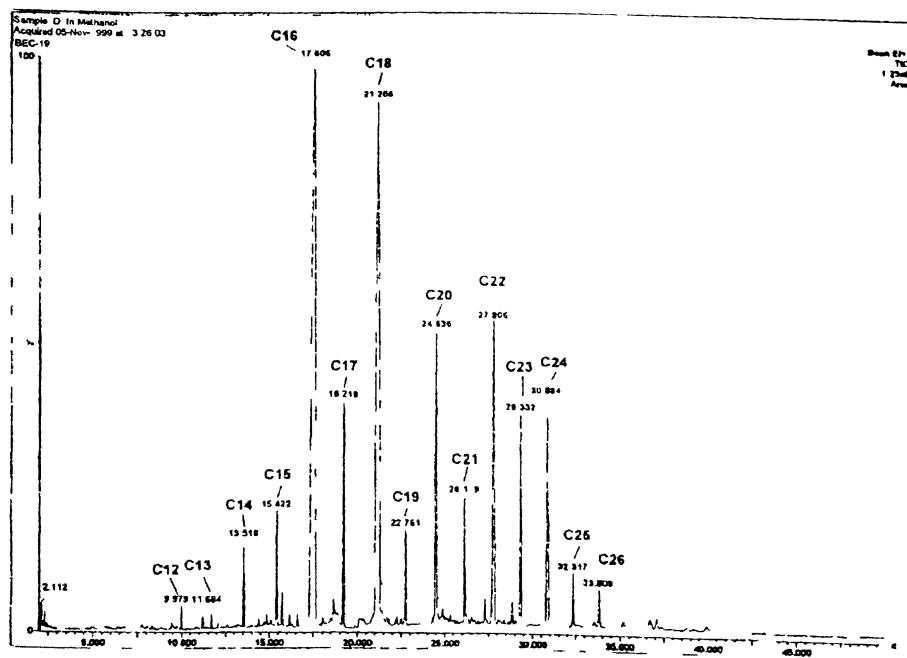


Fig 1 The gas layer chromatogram of the methyl esters of the fifteen saturated fatty acids (with $C_{11}H_{23}CO_2H$ to $C_{25}H_{51}CO_2H$ from *Acanthus ilicifolius* L.)

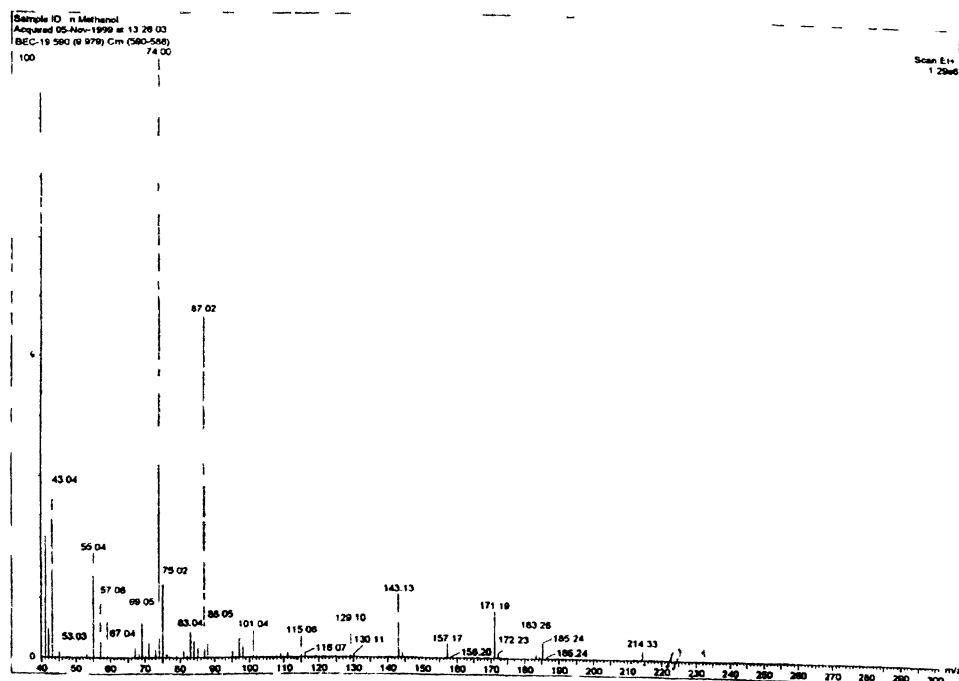


Fig. 2a GC Mass spectrum of $C_{11}H_{23}CO_2CH_3$ (M^+ 214.33)

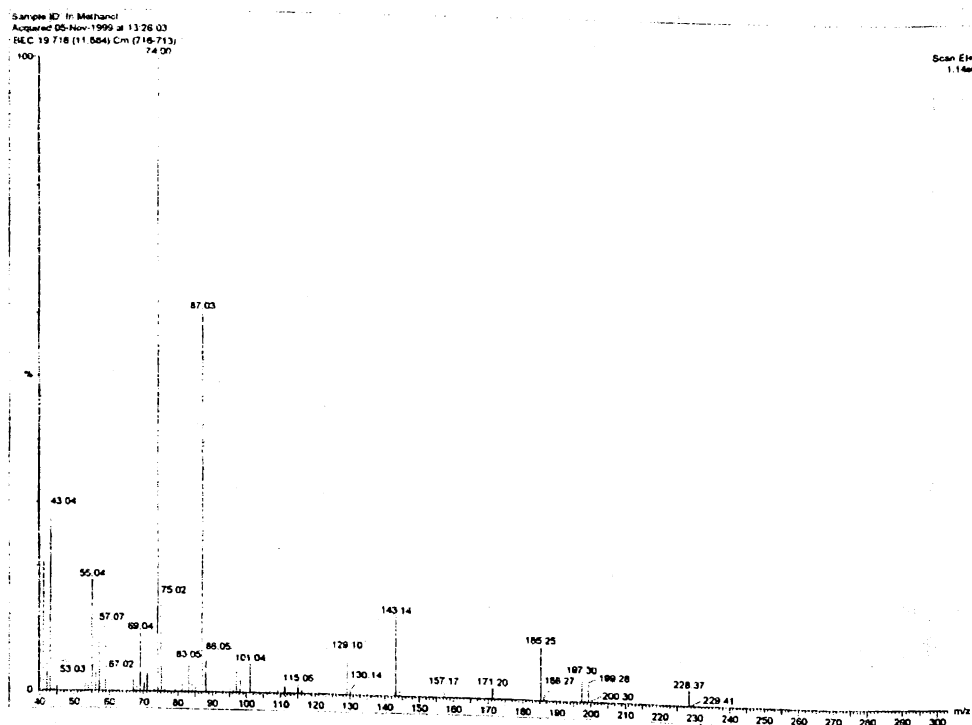


Fig. 2b. GC Mass spectrum of $C_{12}H_{25}CO_2CH_3$ (M^+ 228.37).

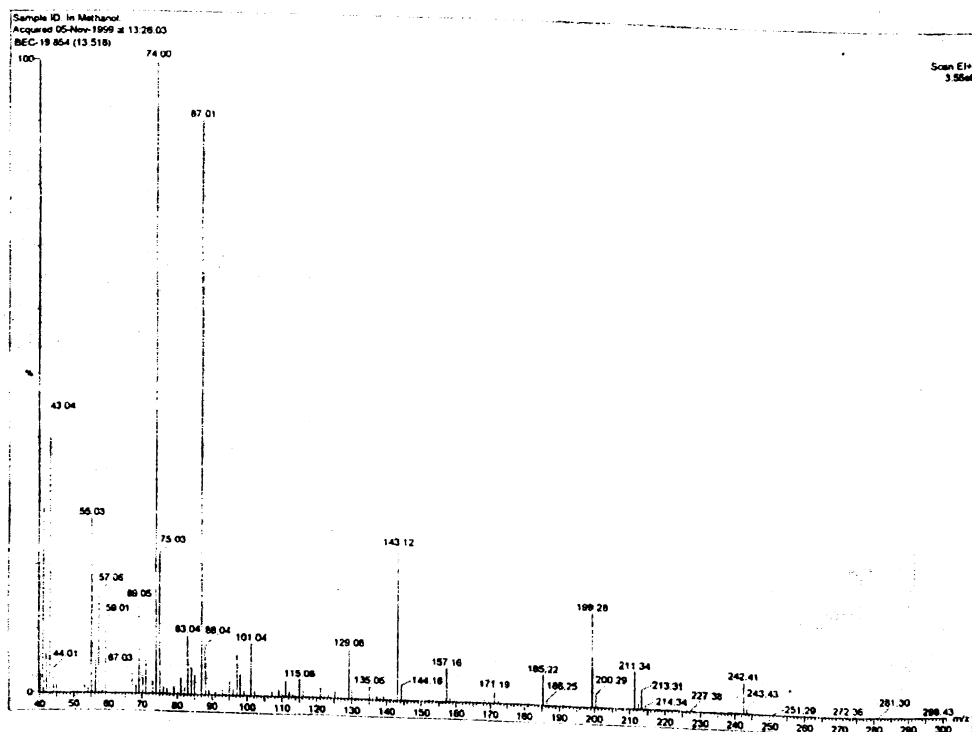


Fig. 2c. GC Mass spectrum of $C_{13}H_{27}CO_2CH_3$ (M^+ 242.41).

Note

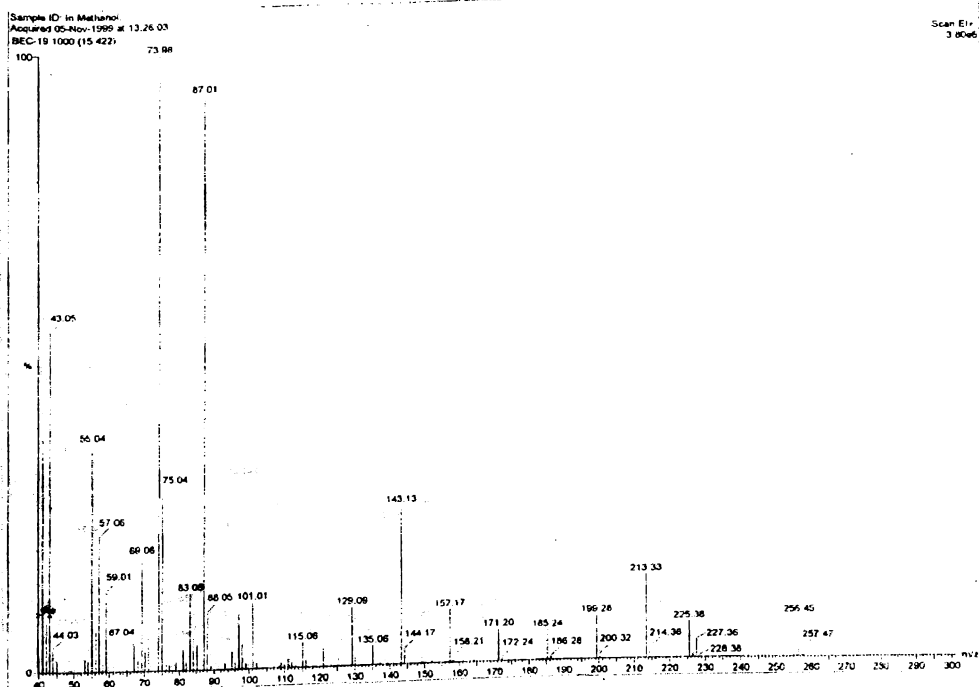


Fig. 2d. GC Mass spectrum of $C_{14}H_{29}CO_2CH_3$ (M^+ 256.45).

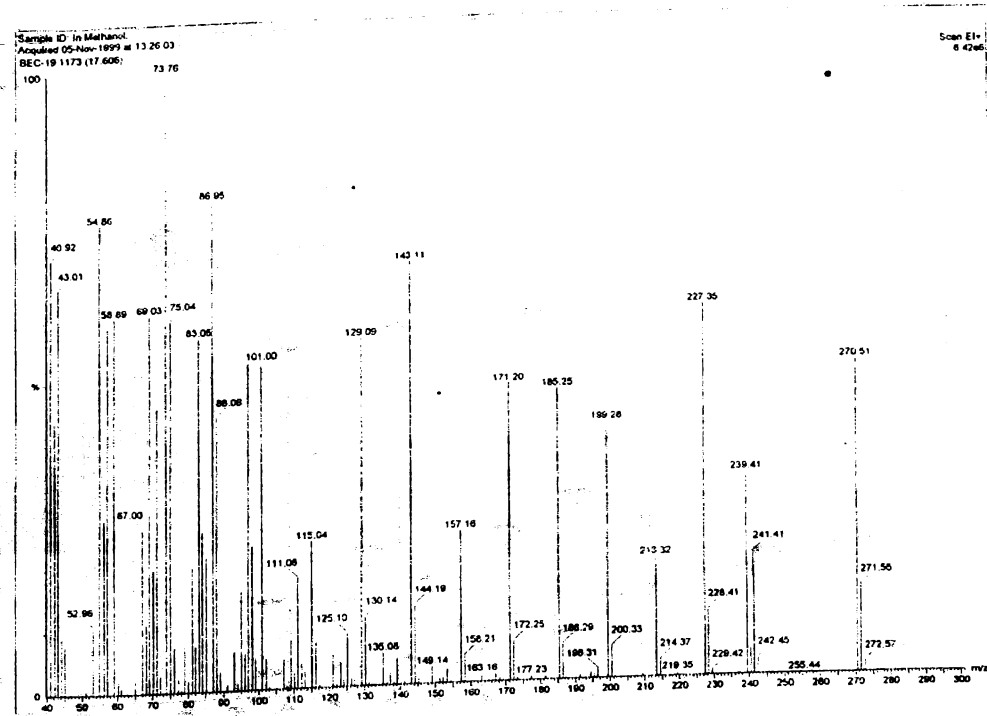


Fig. 2e. GC Mass spectrum of $C_{15}H_{31}CO_2CH_3$ (M^+ 270.51).

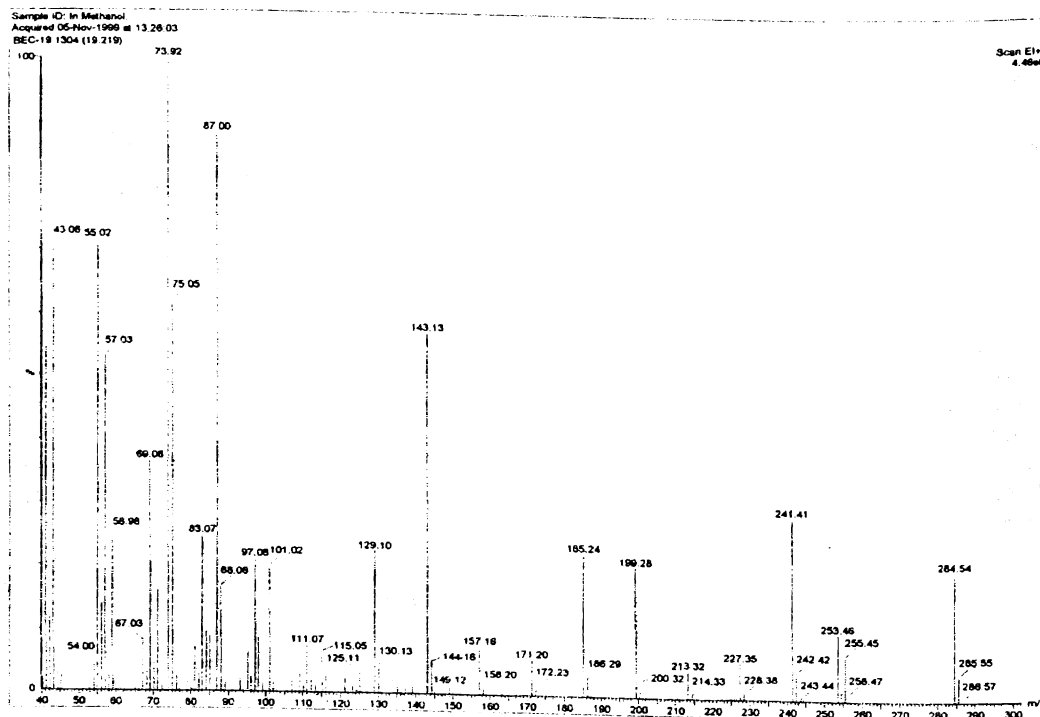


Fig. 2f. GC Mass spectrum of $C_{16}H_{33}CO_2CH_3$ (M^+ 284.54).

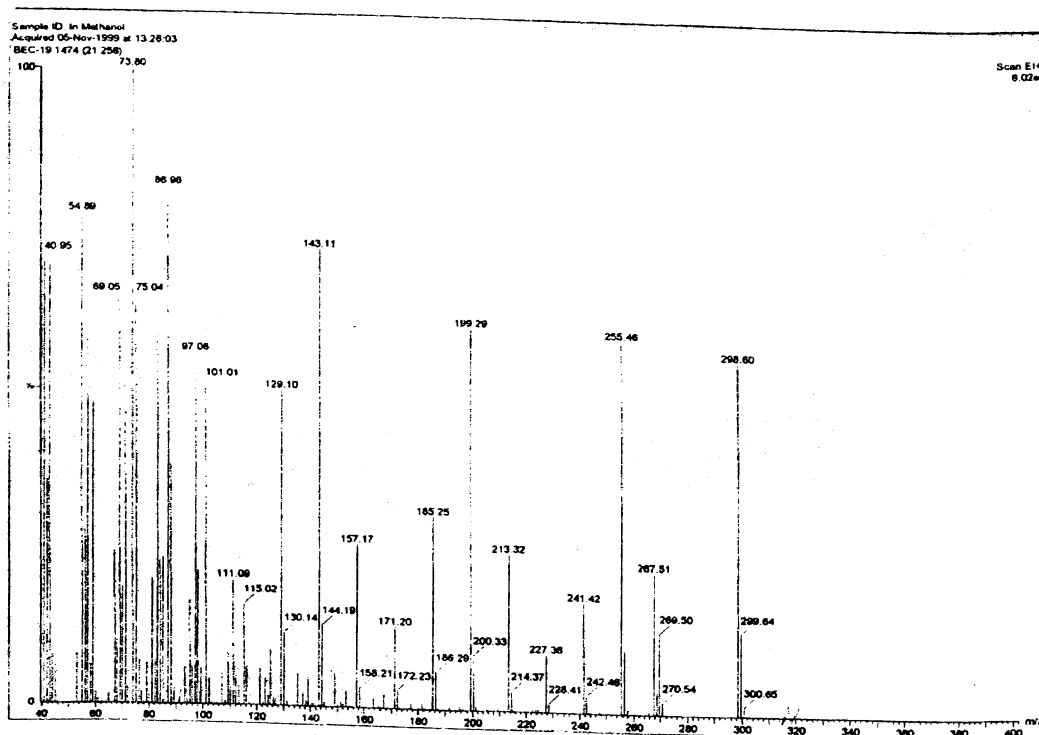


Fig. 2g. GC Mass spectrum of $C_{17}H_{35}CO_2CH_3$ (M^+ 298.60).

Note

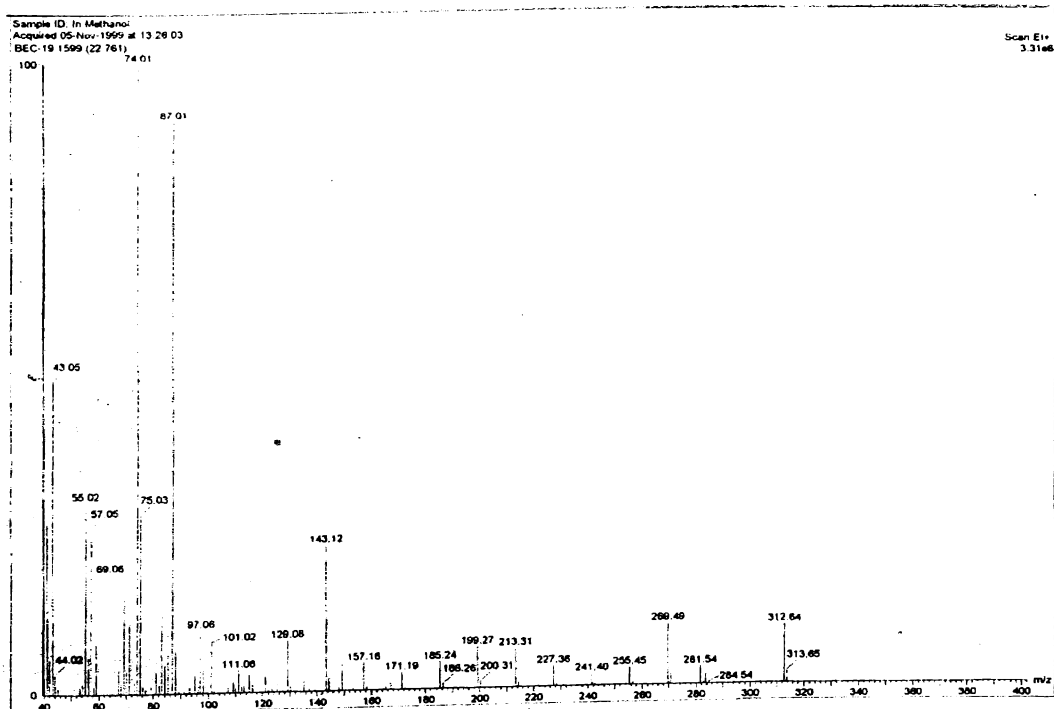


Fig. 2h. GC Mass spectrum of $C_{18}H_{37}CO_2CH_3$ (M^+ 312.64).

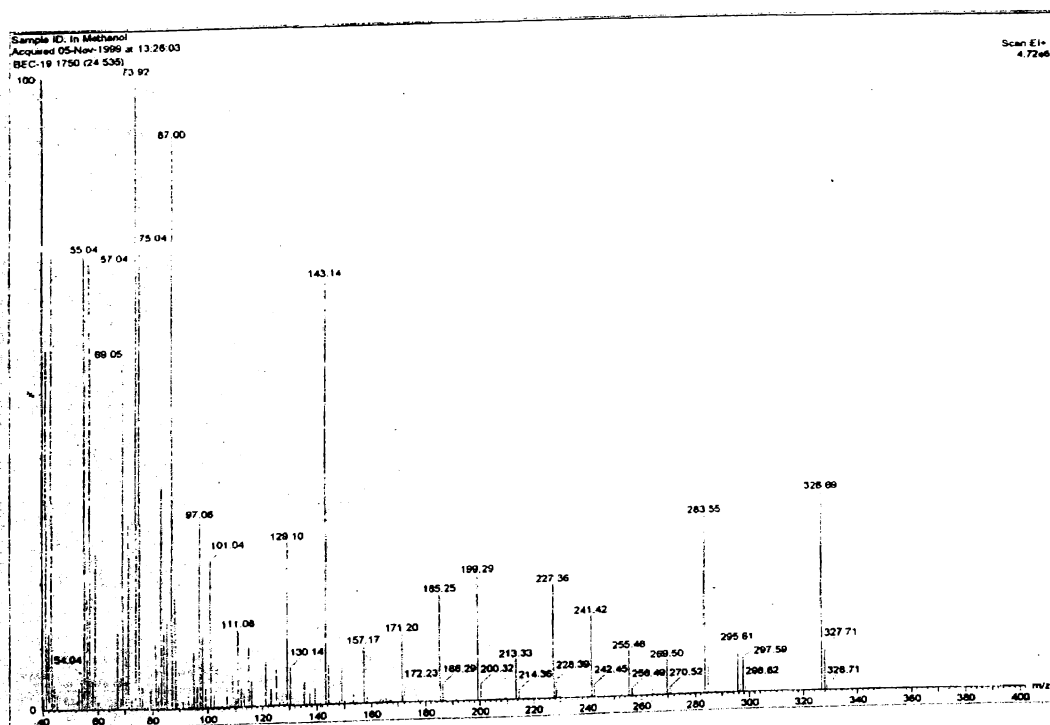


Fig. 2i. GC Mass spectrum of $C_{19}H_{39}CO_2CH_3$ (M^+ 326.69).

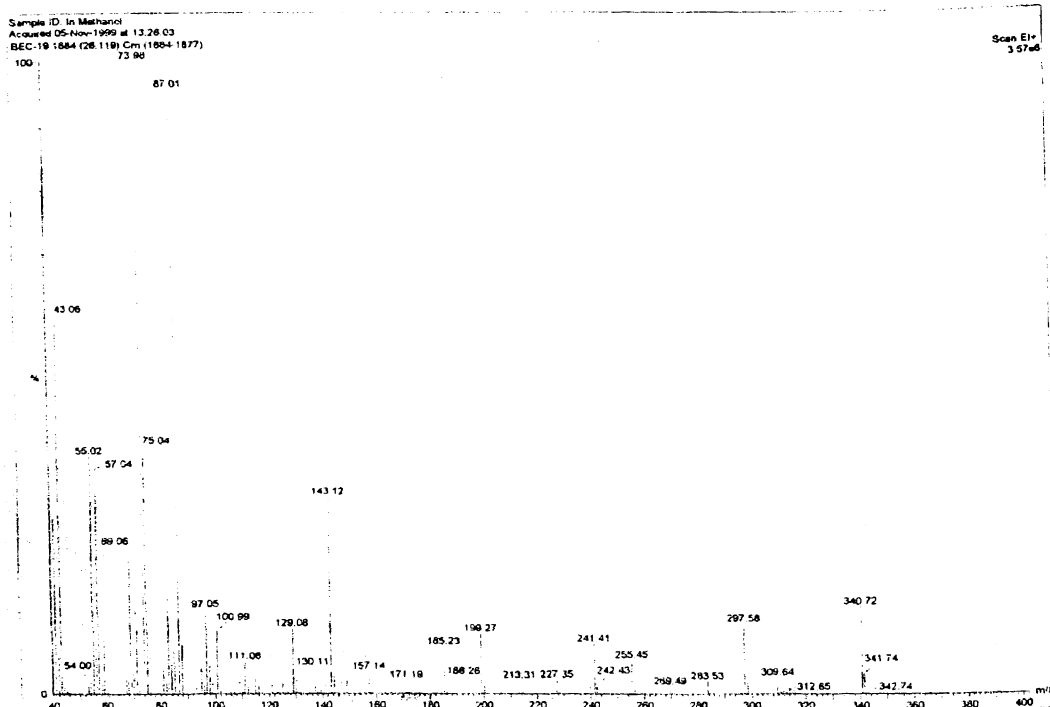


Fig. 2j. GC Mass spectrum of $C_{20}H_{41}CO_2CH_3$ (M^+ 340.72).

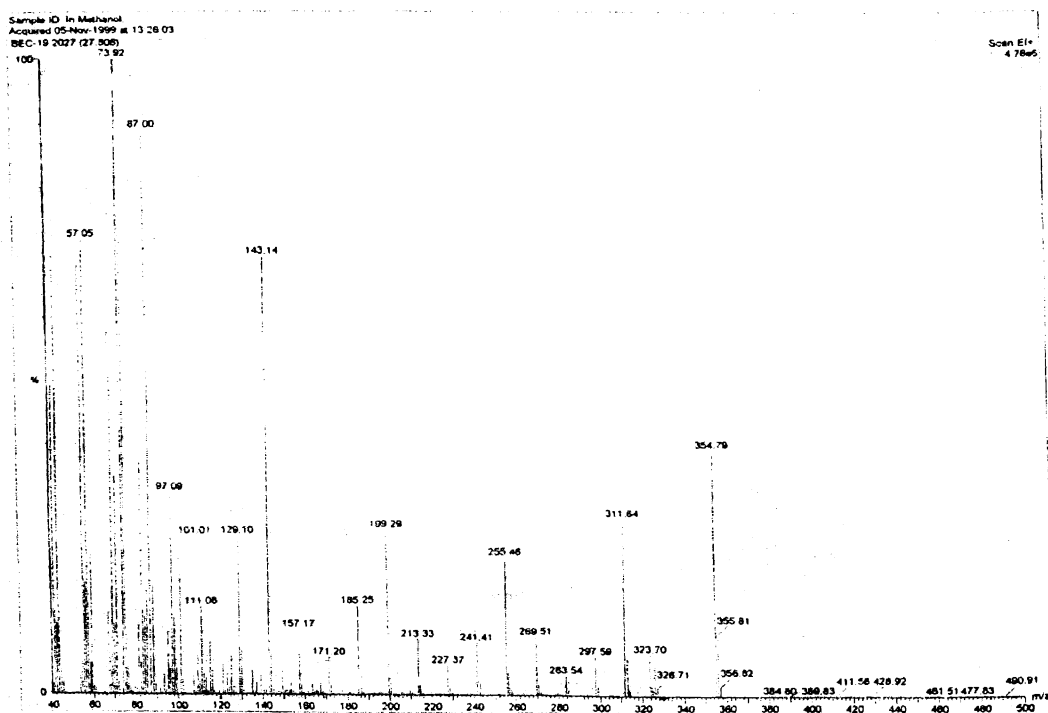


Fig. 2k. GC Mass spectrum of $C_{21}H_{43}CO_2CH_3$ (M^+ 354.79).

Note

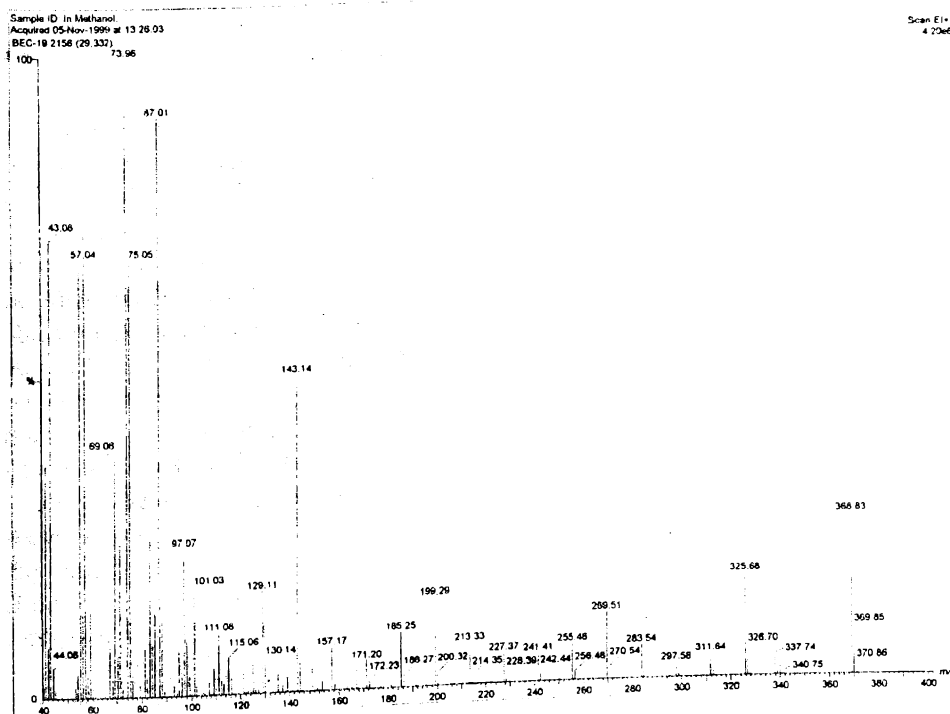


Fig. 2l. GC Mass spectrum of $C_{22}H_{45}CO_2CH_3$ (M^+ 368.83).

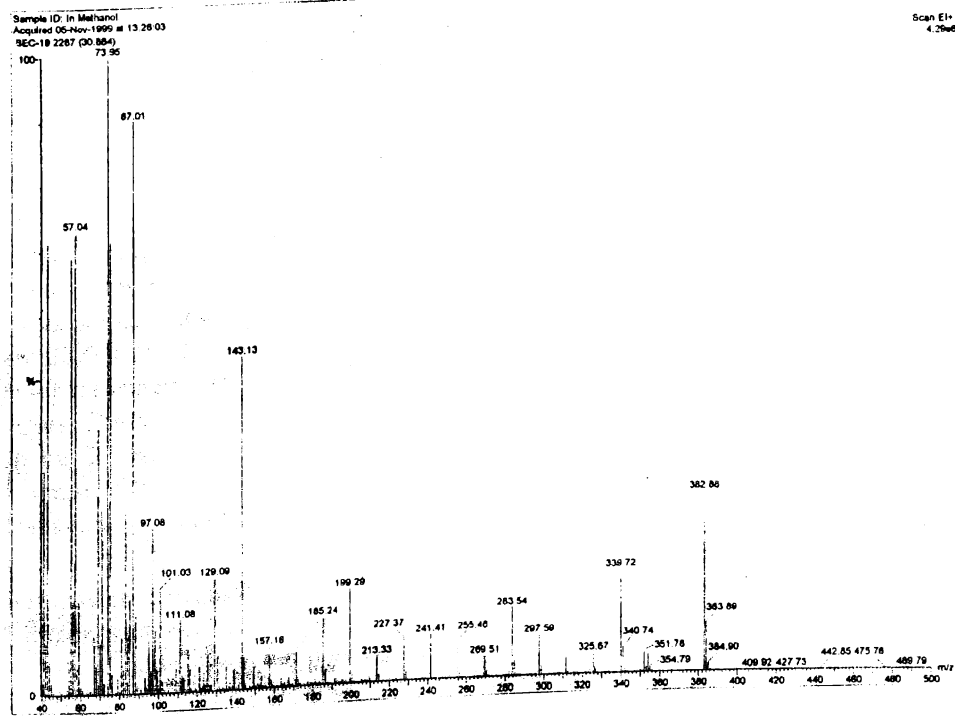


Fig. 2m. GC Mass spectrum of $C_{23}H_{47}CO_2CH_3$ (M^+ 382.88).

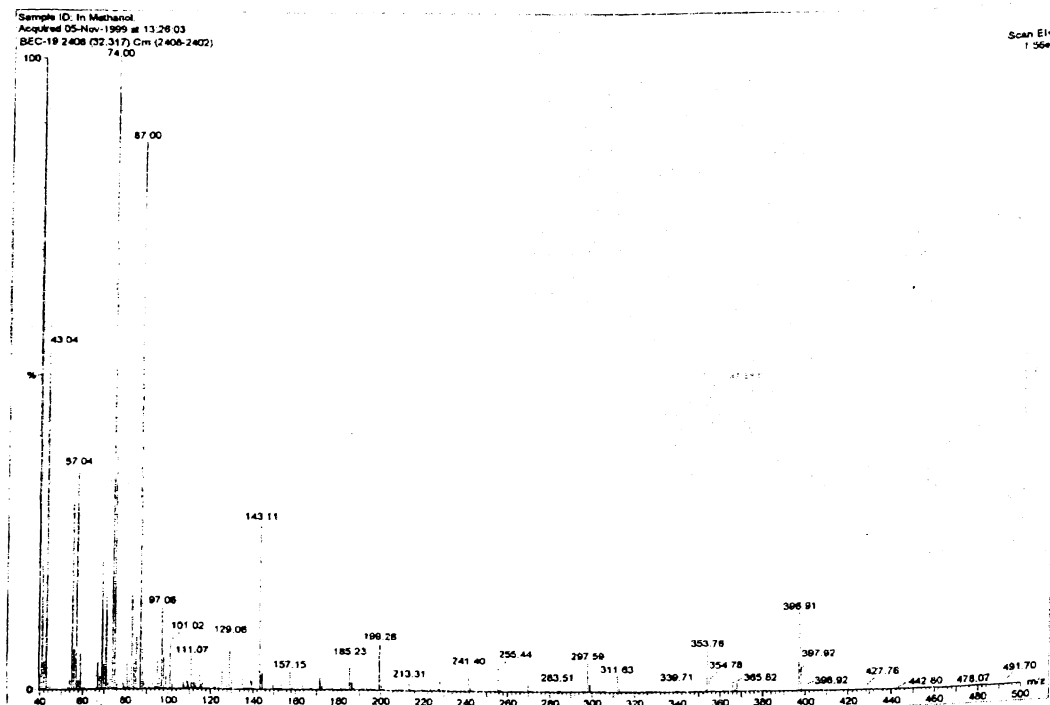


Fig. 2n. GC Mass spectrum of $C_{24}H_{49}CO_2CH_3$ (M^+ 396.91).

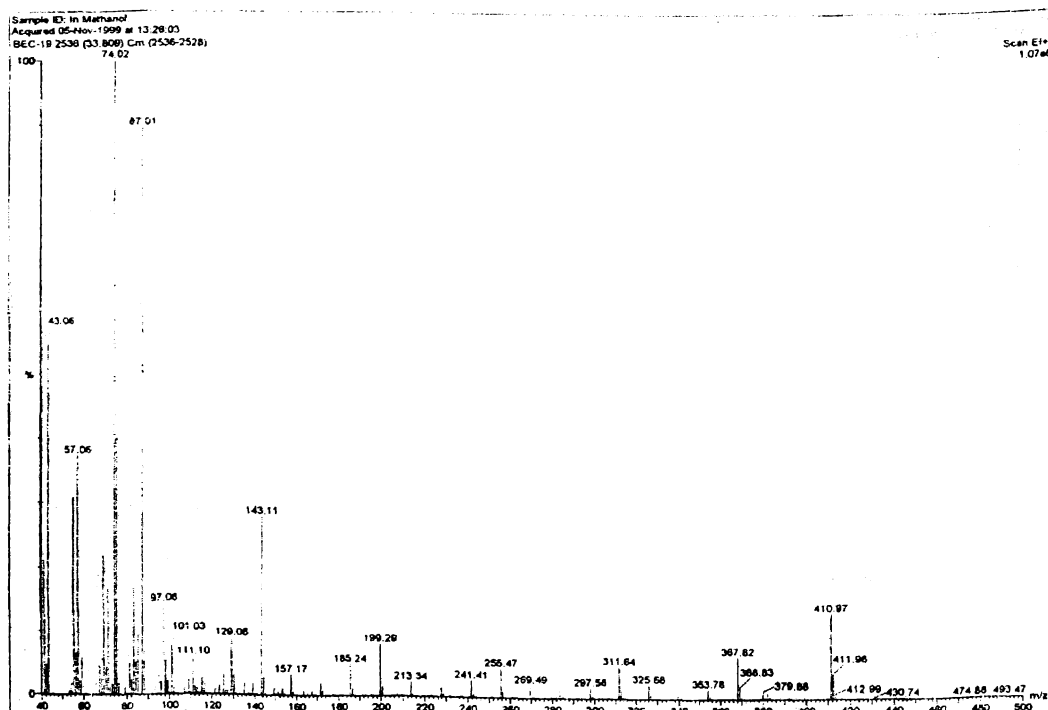


Fig. 2o. GC Mass spectrum of $C_{25}H_{51}CO_2CH_3$ (M^+ 410.97).

chloroform mixture of increasing polarity and also chloroform-methanol solvents was performed. The fractions from petroleum ether-chloroform (60 : 40), chloroform and chloroform-methanol (90 : 10) afforded the crude acid mixture characterized by IR spectrum ($3000\text{--}2800\text{ cm}^{-1}$) for carboxylic hydroxyl, 1704 cm^{-1} for C=O stretching and 710 and 680 cm^{-1} for methylene rocking vibration). The fractions were mixed, concentrated and then dissolved in ether which was then extracted with aqueous sodium bicarbonate solution (5%). The aqueous part on acidification afforded a precipitate suggesting its acid nature which was again extracted with ether and washed with water followed by drying to give only the fatty acids. This fatty acid mixture in dry ether solution containing no other type of compounds was then chromatographed over silica gel with petroleum ether and petroleum ether-benzene fractions (95 : 5). The fatty acid mixture was then methylated by treatment with diazomethane in ethereal solution to afford a mixture of the methyl esters of the fatty acids. The NMR spectra of the methyl esters of fatty acids showed the CH_3 signals at δ 3.5 along with the peaks at δ 2.2 (t) and 1.5 (m), respectively, for the methylene peaks α and β to the ester-carbonyl. The other methylene protons appear at δ 1.04–1.42 and the terminal methyl protons appear at δ 0.82. The gas layer chromatogram of the methyl esters of these saturated fatty acids is shown in Fig. 1.

The mass spectra of all the fatty acid methyl esters separated by GC analyses are shown separately in Figs. 2a–2o (fifteen mass spectra) which distinctly show the continuous presence of each saturated fatty acid followed by its analogue from C12 to C26 at the equal intervals of retention time.

We have also made the quantitative measurement of some of the fatty acids from some of our available standard fatty acids where we have found the % composition of C14 is 2.2; C16, 28.6; C18, 21.5; C20, 4.7; C22, 7.4. Other acids were not measured due to unavailability of their standard samples. The separation⁶ of the mixture of the fatty acid esters by GC technique is successfully done as the proof-of-presence of the homologous series of fifteen fatty acids containing all these even and odd acids together.

The first natural existence of such a complete series (homologous) of fifteen saturated fatty acids in a plant containing both even and odd fatty acids together is of biogenetic significance^{7–9}. Fatty acids in biological systems usually contain an even number of carbon atoms⁴. The biosynthesis of fatty acids comprises a complex se-

ries of integrated reactions⁸. Fatty acids are usually synthesized by acetyl CoA⁷. Elongation by fatty acid synthase stops upon the formation of palmitic acid. All the carbon atoms of fatty acids containing even numbers are derived from acetyl-CoA⁹ and malonyl-CoA as the two carbon donor in the elongation of acyl-CoAs. The longer fatty acids are synthesized by other enzyme systems with fatty acyl-CoAs as the substrates⁹. The synthesis of odd carbon fatty acids starts from propionyl-CoA⁹.

In conclusion, the GCMS spectral evidence as the proof-of-presence of naturally occurring odd and even saturated fatty acids together in a homologous series of fifteen acids in the same plant is an important and biosynthetically interesting report for the first time to our knowledge.

Experimental

General experimental procedures : Instrument used : Fisons instrument (GC 8000 series, MD 800). Oven temperature was programmed from 500 to 1400 C at the rate of 150 C/min, from 1400 to 2500 C at the rate of 50 C/min and holding at 2500 C for 17 min. Column used : DB-5 MS, Capillary column (J and W Scientific, U.S.A.), length 30 mm, internal diameter : 0.25 mm, Helium flow rate : 1.5 ml/min, injector used : split/split-less injector. Source of biological materials : Rural Howrah district, West Bengal, India.

Acknowledgement

We thank Dr. Debi Chakravarti, Emeritus Professor, Government of West Bengal, Bethune College, Kolkata for her interest in the work. We appreciate the help of Professor Parul Chakraborty and Professor Amitava Ghosh of Bose Institute, Kolkata and P. C. Mazumder of Indian Institute of Chemical Biology, Kolkata for providing us some of the standard fatty acids and also Mr. A. Banerjee of Indian Jute Industries' Research Association, Kolkata for the GCMS instrument. We also thank Dr. Reshmi Mukherjee in our laboratory for her help.

References

1. J. D. Hooke, "Flora of British India". B. Singh and M. Pal Singh, Dehradun, India, 1983, Vol. IV; K. R. Kirtikar and B. D. Basu, "Indian Medicinal Plants", B. Singh and M. Pal Singh, India, 1998, Vol. III, p. 1874; R. N. Chopra, S. L. Nayar and I. C. Chopra, "Glossary of Indian Medicinal Plants", C.S.I.R., New Delhi, India, 1998; P. K. Minocha and K. P. Tiwari, *Phytochemistry*, 1981, 135; T. Kanchanapoom, M. S. Kamel, R. Kasai, K. Yamasaki, C. Picheansoonthon and Y. Hirage, *Phytochemistry*, 2001, 56, 369; T. Kanchanapoom, M. S. Kamel,

- R. Kasai, K. Yamasaki, C. Picheansoonthon and Y. Hirage, *Phytochemistry*, 2001, **56**, 135.
2. Daily administration of the leaf extract of *Acanthus ilicifolius* suppresses *in vivo* neoplastic transformation in a transplantable murine Ehrlich-ascites carcinoma model by M. Chatterjee, S. P. Goswami, B. N. Chatterjee, T. Chakraborty, P. Suresh Kanna, C. B. Mahendra Kumar and D. Chakraborty, communicated to *Neoplasma*.
3. S. P. Goswami, K. Ghosh and S. Dasgupta, *Tetrahedron*, 1996, **52**, 12223; S. P. Goswami and K. Ghosh, *Tetrahedron Lett.*, 1997, **38**, 4503; S. P. Goswami and A. K. Mahapatra, *Supramolecular Chem.*, 1999, **11**, 25; S. P. Goswami, K. Ghosh and S. Dasgupta, *J. Org. Chem.*, 2000, **65**, 1907; S. P. Goswami, K. Ghosh and R. Mukherjee, *Tetrahedron*, 2001, **57**, 4987.
4. R. Robinson, "The Structural Relations of Natural Products", First Weizmann Memorial Lectures, 1953, p. 4.
5. A. T. James and A. J. P. Martion, *Biochem. J.*, 1952, **50**, 679.
6. F. R. Cropper and A. Heywood, *Nature*, 1953, **132**, 1101; R. G. Ackman, "Gas-liquid Chromatography of Fatty Acids and Esters", ed. J. M. Lowenstein in "Methods in Enzymology", Academic Press, New York, 1969, Vol. 14, p. 329; F. P. Woodford, "Gas-liquid Chromatography of Fatty Acids and Esters", ed. K. S. Markley in "Fatty Acids", Interscience, 1964, Vol. 3, p. 2249; S. Mishra, A. Ghosh, In: H. F. Linskens and J. F. Jackson (ed.), "Modern Methods of Plant Analysis", Springer-Verlag, New Series, 1991, Vol. 12, p. 218.
7. P. R. Vagellog in "Biosynthesis of Saturated Fatty Acids in Biochemistry of Lipids", ed. T. W. Goodwin, MTP International Review of Sciences : Biochemistry, Series One, Butterworths, London Univ. Park Press, Baltimore, 1974, Vol. 4, p. 110.
8. D. E. Green and S. J. Wakil in "Lipid Metabolism", ed. K. Bloch, John Wiley, New York and London, 1960, p. 1.
9. A. Kawaguchi and N. Iwamoto-Kishara A. Satu in "Comprehensive Natural Product Chemistry", eds. D. Barton and K. Nakanishi, Elsevier, Amsterdam-Lausanne, New York, Oxford-Shannon-Singapore-Tokyo, 1999, Vol. I, p. 23.