

Isolation of Docosyl Docosanoate from *Fagonia cretica* Linn

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ABSTRACT. Hexane extract of the air-dried whole plant (*Fagonia cretica* Linn) afforded a crystalline compound, m.p. 75-76°C, which has been identified to be docosyl docosanoate. The molecular formula of this ester has been determined mass spectrometrically and by combustion analysis, while the structure has been assigned on the basis of I.R. and NMR spectroscopic techniques. The compound on alkaline hydrolysis yielded docosanoic (behenic) acid and 1-docosanol, which confirmed the structure of this ester.

Fagonia cretica Linn., of the family of *Zygophyllaceae* is a small green spiny under-shrub with stiff erect grandular branches, and is widely distributed through out Pakistan. It is known as "dhamasa" in Sind and "suchi booti" in Punjab. In the traditional system of medicine it has been used for various ailments (Chopra *et al.* 1958, Kashyap and Joshi 1936).

Several investigations of the constituents of *Fagonia* species (*Zygophyllaceae*) have been reported (Ehsanul Haq and Hussain, 1965, Hussain *et al.* 1966; Tiwari *et al.* 1966, Amjad and Zahida 1966 & 1967, Ahmed *et al.* 1969, Rimpler and Rizk, 1969; Rizk *et al.* 1972; Attaur-Rehman *et al.* 1982 and Khan *et al.* 1972). The present work deals with the hexane extract of *F. cretica* Linn.

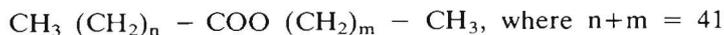
Results and Discussion

The majority of phytochemical studies on *Fagonia cretica* Linn, were mainly concerned with alkaloidal, terpenoid and saponin components of this plant (Tiwari

et al. 1966; Amjad and Zahida, 1966 & 1967 and Ahmed *et al.* 1969). A few of the investigations deal with higher alcohols, free proteins and amino acids (Tiwari *et al.* 1966). However, very limited work has been carried out on the lipids and fatty acids of the plant (Saeed *et al.* 1976). As a result of the present work on the n-hexane extract of this whole plant, a compound (0.1% of the weight of powdered sample), aliphatic in nature, having no nitrogen or other elements except oxygen and melting sharply at 75-76°C was obtained. This turned out to be the major constituent of this extract. The combustion analysis corresponded to $C_{22}H_{44}O$. However, the mass spectrum of the purified sample showed molecular ion absorption corresponding to m/z 648 (5.6%) which agrees with the molecular formula $C_{44}H_{88}O_2$.

The IR spectrum of the sample exhibited a strong absorption at 1740 cm^{-1} , which may be attributed to the stretching vibration of the carbonyl ester. This was further confirmed by a prominent absorption at 1175 cm^{-1} , most likely due to an adjacent C-O stretching vibration. Since no absorption was observed in the regions $900\text{-}750\text{ cm}^{-1}$ and $3100\text{-}3000\text{ cm}^{-1}$ the absence of any aromatic C-H ring vibration may be suggested. Thus, the aliphatic nature of the ester can be inferred. The absorption at 720 cm^{-1} may be attributed to repeating methylene units. The ^1H NMR spectrum confirmed the absence of any aromatic protons. It showed a triplet at $\delta 0.88$ ppm suggesting the presence of terminal methyls (attached to methylenes) and a complex multiplet between $\delta 1.20\text{-}1.70$ ppm suggesting the presence of repeating methylene units in the molecule and substantiating the suggestion derived from IR spectrum regarding repeating methylene units in the molecule. A triplet at $\delta 2.85$ ppm was observed in the spectrum, which can be attributed to a proton of carbon directly attached to a carbonyl functionality in a $-\text{CH}_2-\text{CO}-$ group. The spectrum also signalled an other triplet at $\delta 4.55$ ppm, which may be assigned to proton of carbon directly bonded to oxygen in a $-\text{CH}_2-$ group. The integral showed these protons in the ratio 3:40:1, in agreement with the proposed formula.

The fully decoupled C^{13} NMR spectrum further confirmed the absence of any resonance peak in the aromatic region. It further displayed the characteristic carbonyl resonance at $\delta 173.95$ ppm (singlet) and a singlet at $\delta 64.41$ ppm accounting for a $-\text{O}-\text{C}-$ carbon resonance. This along with information from the IR and ^1H NMR spectra, confirmed the presence of an aliphatic ester functionality. In addition C^{13} NMR spectrum displayed a resonance peak at $\delta 14.10$ ppm (singlet), typical of a terminal methyl group and numerous peaks in the region $\delta 22.0\text{-}34.0$ ppm (as indicated in the experimental section) possibly for repeated $-\text{CH}_2-$ units. The attached proton test (ATP) established the peak at $\delta 14.10$ ppm as a methyl carbon and peaks in the region $\delta 22.0\text{-}34.0$ ppm as a number of methylene carbons. All the above data strongly suggested the product to have the following structure:



The alkaline hydrolysis of this ester afforded docosanoic (behenic) acid and 1-docosanol (behenyl alcohol). The IR spectrum of an authentic sample of docosanoic acid overlapped with the similar spectrum of the acid obtained from the hydrolysis reaction, and their mixed melting points did not show any depression. Similarly the m.p. of the alcohol (80-81°C) isolated corresponded to the m.p. of 1-docosanol (Dimter, 1941). Its IR spectrum was identical with IR spectrum of this alcohol in the Sadtler Index (IR No. 15646) (The Sadtler standard spectra). It was further confirmed through the formation of phenylurethane derivative. Thus, the ester obtained from the hexane extract of *Fagonia cretica* Linn., was confirmed to be docosyl docosanoate. The presence of this ester in this plant is being reported for the first time. However, the corresponding unsaturated ester obtained from the marine source (*Actinosola*) on hydrogenation has been reported to yield this ester (Bergmann *et al.* 1956).

The mass spectrum of this ester showed main absorptions corresponding to m/z 648 (5.6%), m/z 341 (36 %), m/z 313 (100%), m/z 257 (26.5%), m/z 125 (26%), m/z 111 (40%), m/z 97 (61%), m/z 71 (56.5%), and m/z 57 (68.6%). The absorption at m/z 648 (5.6%), results from the molecular ion of docosyl docosanoate, which is considered to undergo characteristic McLafferty rearrangement in agreement with fragmentation pattern of other long-chain esters (Silverstein *et al.* 1981) to afford the cation (m/z 341; 36%). This is followed by initial loss of an ethene molecule to afford the most abundant cation (m/z 313; 100%), which in turn may lose an other neutral molecule, butadiene, to afford the cation (m/z 257; 26.5%). The remain cations, as mentioned in the experimental section, may be resulting from the cleavage of C-C bond with retention of charge on the oxygen-containing fragments or on the alkyl fragments (Silverstein *et al.* 1981).

Experimental

Melting points were determined in capillaries on Gallenkemp, melting point apparatus, and were uncorrected. The IR spectra were run on Beckman IR 5A and Beckman Acculab. 10 spectrophotometer and ^1H NMR spectra were recorded on Hitachi R-24B high resolution NMR spectrometers.

n-Hexane, ethylacetate, 2-propanol and sodium hydroxide used were analytically pure.

Extraction Procedure

Crushed and powered sample (5 Kg) of the whole plant, which was dried under sun-shade, was taken in n-hexane (10 l) and occasionally stirred. After a

week the contents were filtered and the solvent removed from the filtrate on a water bath under reduced pressure, which left a residue (5 g; 0.1%). This residue deposited crystals after a lapse of a week, which were separated and repeatedly crystallised from n-hexane affording a compound m.p. 75-76°C. The process was repeated several times to collect sufficient amount for its characterisation.

Its IR (KBr disc) showed main absorptions at 2900, 2850, 1740, 1460, 1175, 730 and 720 cm^{-1} . Its mass spectrum showed major absorptions at m/z 648 (5.6%), 341 (36%), 313 (100%), 257 (26.5%), 125 (26%), 111 (40%), 97 (61%), 83 (52%), 71 (56.5%) and 57 (68.6%). The elemental analysis of the compound indicated only the presence of carbon (81.6%) and hydrogen (13.7%). No nitrogen was detected in it.

It ^1H NMR showed signals at 0.88 (triplet, CH_3), 1.20-1.70 (complex multiplet, $-(\text{CH}_2)_n-$), 2.85 (triplet, $-\text{CH}_2-\text{CO}-$) and at δ 4.55 ppm (triplet, $-\text{O}-\text{CH}_2-$) in the ratio 3:40:1. The fully decoupled ^{13}C NMR showed absence of any aromatic region, displayed singlets at 173.95 ($-\text{CO}-$), 64.41 ($-\text{O}-\text{C}$), 14.10 (terminal CH_3) and numerous peaks at 22.72, 25.10, 26.01, 28.74, 29.22, 29.32, 29.40, 29.74, 31.97 and at δ 34.47 ppm (all singlets possibly for repeated $-\text{CH}_2-$ groups).

Alkaline hydrolysis

The compound (0.5 g) was added to a solution of sodium hydroxide (200 mg) in 2-propanol (10 ml), refluxed for two hours and thereafter the alcohol was distilled off. Subsequently water (10 ml) was added to it and extracted with ethylacetate. The extract was dried over anhydrous sodium sulphate to afford white crystalline material (0.165 g; m.p. 80-81°C), which was close to the m.p. of 1-docosanol (Dimter, 1941), and its IR spectrum agreed well with IR No. 15646 (The Sadtler standard spectra). It was further confirmed through phenyl urethane derivative. In this connection equal weights of the alcohol and freshly distilled phenylisocyanate were heated to 100°C for 30 minutes. The excess reagent was removed under vacuum and docosyl phenyl-urethane was crystallised from pentane having m.p. 87°C (lit. m.p. 87°C, Bergmann *et al.* 1956).

The aqueous part having junction residue was acidified with dilute hydrochloric acid and extracted with n-hexane (10 ml \times 3). The extract was washed with water and dried over anhydrous sodium sulphate. Its filtrate left behind a residue (0.172 g), which was crystallised from n-hexane to afford a compound m.p. 79-80°C. Its IR spectrum overlapped with IR spectrum of an authentic sample of docosanoic acid and their mixed m.p. did not show any depression.

Acknowledgement

The authors are grateful to Professor Alan R. Katritzky and Barbara Nowak

Wydra of Chemistry Department, University of Florida, Gainesville, USA, for assistance in running ^{13}C NMR and mass spectra, and liberal discussion regarding structure elucidation of the compound.

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(Received 05/12/1987;
in revised form 28/07/1988)

فصل مركب ديكوسانوات دوكوسايل من نبات فاجونيا كريتيكا لين

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أعطي مُستَخْلَص الهكسان للنبات الكامل المجفف بالهواء (نبات فاجونيا كريتيكا لين)، مركباً بلورياً درجة انصهاره ٧٥ - ٧٦ سيلزية، ولقد ثبت أن هذا المركب هو دوكوسانات دوكوسايل، وقد تم تحديد الصيغة الجزيئية لهذا الاستر باستخدام طيف الكتلة والتحليل الاحراقي، أما التركيب فقد تحدد على أساس من طيف الأشعة دون الحمراء والرنين النووي المغنطيسي. وعند حلماًة الاستر في وسط قاعدي انتج حمض ديكوسانويك (بهنيك) بالاضافة إلى ١ - ديكوسانول، مما عزز تركيب هذا الاستر.