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INVESTIGATION OF THE FREE STEROID FROM PALMYRAH(*Borassus flabellifer* L.) FRUIT PULP

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Abstract

The study describes the isolation of a Steroid from palmyrah fruit pulp(PFP) by MPLC. Spectroscopic studies using ^{1}*H-NMR and* ^{13}*C-NMR showed its structure to be* β *Sitosterol. The steroid was isolated from PFP by extracting with methanol, cleaning with petroleum ether followed by desugaring with dry cellulose chromatography and separated by medium pressure liquid chromatography (MPLC). Mass spectrometry shown a molecular weight of 414. The main problem of whether the ethyl group in position 24 was* α *or* β *was deduced from study of the literature and data banks of* ^{13}*C-NMR. This was confirmed by* ^{1}*H-NMR data.*

keywords :Palmyrah, Steroid, Sitosterol, MPLC, ^{1}H-NMR, ^{13}C-NMR

1 Introduction

Palmyrah is common palm growing in the arid regions of the country. It is estimated that ten million mature trees are available. Most parts of the tree are used, but there is estimated 15 *kt.* palmyrah fruit pulp per annum, which is underutilized because it is bitter[1].

The bitter principle is a steroidal saponin, which Jeyaratnam[2] had given the name Spirost- 5en- 3βol(24R).
It is now clear that a common steroid is the aglycone for all the flabelliferins of PFP (Ariyasena D.D., 2001 Private communication) and is not spirost -5en- 3βol as stated by Jeyaratnam[2]. The bitter principle F-II (steroidal tetraglycoside)[3] and two inactive flabelliferins F_C (steroidal triglycoside) and F_D (steroidal diglycoside) have been isolated and studied[4][5].
The objective of this study was to clarify the controversy in the literature regarding the structure of the free steroid. In this study the isolation of the steriod and its structural eluicidation by ^{13}C and ^{1}H- NMR is reported.

2 Methods and materials

The palmyrah fruits were collected from the Hambantota district. The pulp was extracted manually in the laboratory. The fruit pulp portions (200 g) were extracted with methanol (200 ml), fats and carotenoids extracted with petroleum ether 60-80^{0}C (320 $ml\times$ 2) and concentrated following the procedure reported previously[3]. This extract also contained sugars[2][3][6] which were removed using dry cellulose chromatography[3].

2.1 Apparatus

The medium pressure liquid chromatography set up, consisted of a solvent pump (FMI lab pump, Model QD-O-SSY from Fluid Metering Inc. Oyster Bay N.Y., U.S.A., $\frac{1}{8}$ $inch$ piston diameter; pressure up to 100 PSIG , flow range 0 to 100 ml/min); SEPARO columns, (variable length) (Baeckstrom SEPARO AB, Larsbergsvagen 24, SE 18139 Lidingo, Sweden); Teflon tubes between the pump and the column were intersected with luerlock connectors to make sample injection possible with a luerlock syringe; a constant volume mixing chamber combined with a reservoir to create continuous gradients; a Gilson model 201 fraction collector; a variable wavelength UV absorbance detector (ERMA optical works Ltd, ERC-7210 model); a PM 8251 single pen paper recorder (PHILIPS).

2.2 Packing material

The columns (6 cm length and column diameter 1.5 cm) were dry packed with Merck silica gel 60 A, particle size 40-63 μ, for column chromatography. The column bed was compressed by axial compression (pressure was 8 bars) in a quick grip carpenter's vice before use.

2.3 Fitting of the column

The columns were made of glass and all connections between pump, injector, column and detector were made with teflon tubes. Acetone was used to clean all residues of adsorbent from the wall of the column before adjusting the pistons to avoid leakage. The MPLC apparatus was set up as described by Nikawala[6] and packed with silica gel and the pre-adsorbed sample introduced.

2.4 The MPLC technique

The mixture of flabelliferins and steroid weighed accurately (2.331 g) was introduced in 2.5 ml of MeOH (Analar grade) and 2.5 ml of CH_2Cl_2. Merck silica gel 60 A, particle size 40-63 μ (1.5 g), for column chromatography was used. MeOH and CH_2Cl_2 were removed using a rotary evaporator. The trace amounts of solvents were removed using an oil pump. The oil pump was connected to the sample through an ice bath containing dry CO_2 acetone mixture at -71^{0}C and the pressure was balanced by a Hg column. The traces of solvents were removed through this apparatus over a period of 12 *hours*. After which the material was kept in a vacuum desiccator for 48 *hours*. This resulted in a powder which was neither oily nor sticky. As gradients were used, the flow rate was sufficient to dissipate the heat of adsorption released by the analytes and the polar components of the gradients. In experiments, the 1.5 cm i.d. columns were run at a flow rate of 10 ml/min.
The binary mixtures of CH_2Cl_2 and CH_3OH (each portion 20 ml) were prepared by diluting a chosen amount of the polar component (CH_3OH) with the same amount of the non-polar component (CH_2Cl_2) and removing half of the resulting mixture to a bottle (dilution factor 0.5), after which 20 ml of the non-polar component was added to the remaining mixture and so on. This gave equal amounts of the concentrations 50,25,12.5,6.25,3.125 etc. % CH_3OH, except for the last dilution at which twice the amount was used. The gradient of the solvent system used in succession was CH_2Cl_2 100%, and the ratios of CH_2Cl_2 : MeOH were 99.8 : 0.2, 99.6 : 0.4, 99.2 : 0.8, 98.4 : 1.6, 96.87 : 3.13, 93.75 : 6.25, 87.5 : 12.5, 75 : 25, 50 :50, and MeOH 100%. In the procedure 20.0 ml of each of these portions were added to the reservoir as the column was running.
By using the fraction collector 5.0 ml fractions were collected. Fractions were monitored on G_{60} F_{254} prepared plates (100 $_\mu$m) in the solvent system CH_2Cl_2 : CH_3OH, 3 :1 ratio. Spots were visualized using anisaldehyde reagent (Anisaldehyde : Glacial acetic acid :- conc. H_2SO_4, 1 : 100 : 2)

2.5 Mass spectrometry (MS):

MS for determination of steroid structure was obtained using a GC-3400 (Varian); MS (Finnigan mat.) SSQ-7000 mass spectrometer using the Direct Inlet method.

2.6 NMR:

^{1}H-NMR, ^{13}C-NMR and 2DNMR were performed with a BRUKER-DMX 500 at 300^0K in solution of $CDCl_3$ using TMS as an internal reference.

3 Results

3.1 Isolation of free steriod:

The free steriod eluted at 55- 60 *ml* (fraction no 18-20) in the 93.75% CH_2Cl_2 elute and yielded approximately 4.38 *mg* from MPLC.

3.2 Structure of Steriod :

Table 1: Assigned ^{1}H and ^{13}C chemical shifts for sitosterol- 5en-3βol (24αEt)

Carbon	δ_C	δ_H	
1	37.24	1.08	1.86
2	31.65	1.85	1.51
3	71.81	3.55	
4	42.29	2.30	2.25
5	140.74	—	
6	121.72	5.37	
7	31.90	1.52	1.97
8	31.89	1.46	
9	50.11	0.95	
10	36.50	—	
11	21.07	1.49	1.47
12	39.76	1.17	2.02
13	42.31	—	
14	56.75	1.01	
15	24.29	1.56	1.07
16	28.24	1.85	1.27
17	56.04	1.11	
18	11.85		0.70
19	19.39		1.03
20	36.14		1.37
21	18.77	0.94	
22	33.93	1.05	1.35
23	26.04	1.18	
24	45.82	0.93	
25	29.13	1.69	
26	19.02	0.87	
27	19.81	0.83	
28	23.05	0.85	1.26
29	11.97	0.86	

Table 2: Comparison of ^{13}C -NMR data

Clionasterol (24βEt) *	Steroid from this study	Sitosterol(24αEt)**
11.87	11.85	
12.32	11.97	
18.84	18.77	
18.98	19.02	19.07
19.41	19.39	
19.61	19.81	19.84
21.10	21.07	
23.02	23.05	
24.32	24.29	
26.38	26.04	26.13
28.25	28.24	
28.95	29.13	29.18
31.68	31.65	
31.92	31.89	
31.93	31.90	
33.92	33.93	
36.28	36.14	36.17
36.52	36.50	
37.27	37.24	
39.78	39.76	
42.31	42.29	
42.33	42.31	
46.07	45.82	45.85
50.14	50.11	
56.08	56.04	
56.77	56.75	
71.84	71.81	
121.72	121.72	
140.76	140.74	

* Reynolds et al., (1997)
** Wright et al., (1978)

The mass spectrum showed a molecular weight of 414 showing that it was not a stigmast sterol 24Et (Figure 1) ^{1}H-NMR(Figure 2) and ^{13}C-NMR did not indicate a spirost ring system but rather a stigmast ring system as found in sitosterol.
It is speculated that this is the free steriod of Jeyaratnam. The structure has been identified as sitosterol in this study and not a stigmaststerol as stated by Jeyaratnam[2][7]. Studies at the Royal Institute of Technology, Sweden on this steriod gave the following data (Table 1, 2).

4 Disscussion

This was found to be sitosterol. The molecular weight was 414(Figure 1). Therefore the steroid cannot be stigmast-5en-3βol(24αEt)(M.W.412). This fits a sitosterol structure with one less double bond. Chemical shifts in ^{1}H-NMR and ^{13}C-NMR showed one oxygen one olefinic proton and 6 methyl groups. By comparing with data banks for ^{13}C and ^{1}H-NMR the structure was confirmed as a sitosterol (M.W.414) (Table 1). Data from the literature[8][9] showed that the ethyl must be in an α conformation (Table 2). The structure is therefore identical to sitosterol. Sitosterol contains an -OH group at C-3 and a double bond at position 5 and is supported by ^{1}H-NMR and ^{13}C-NMR data.

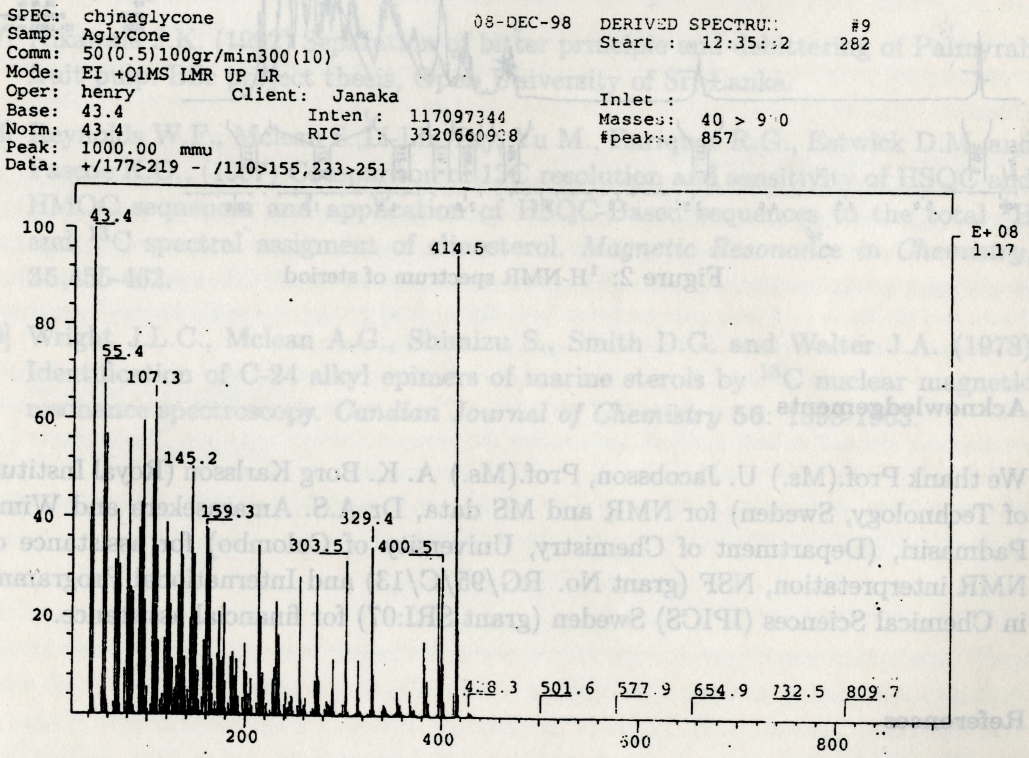

Figure 1: Mass spectrum of steriod

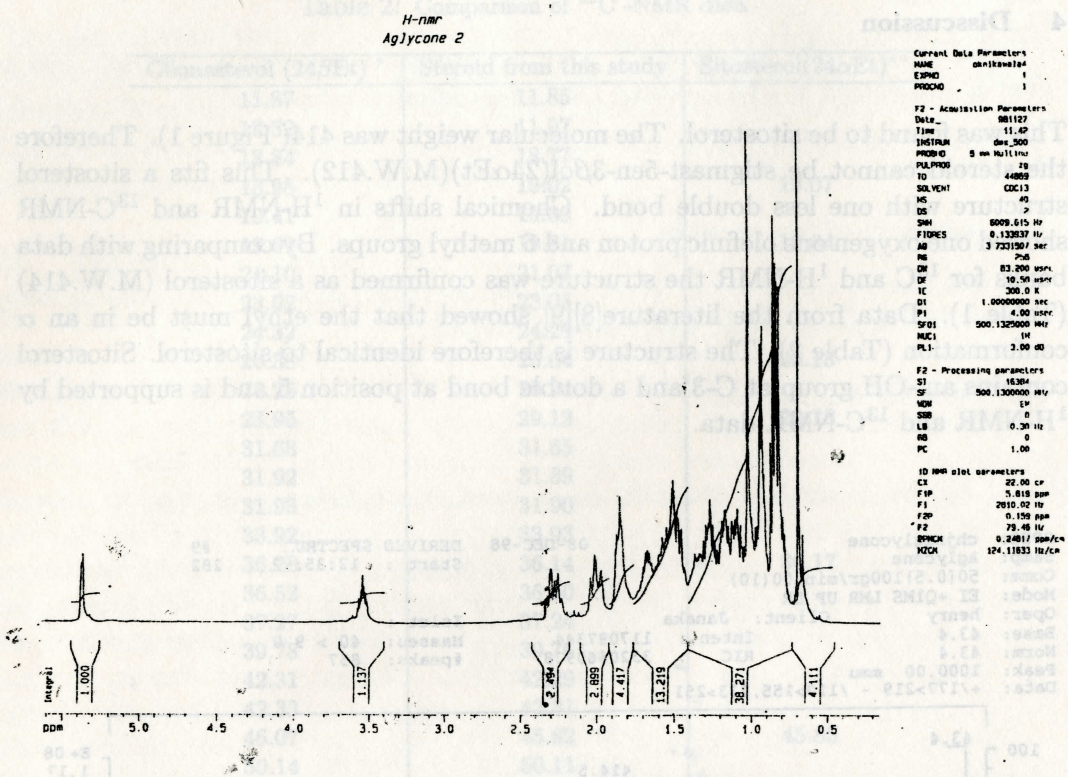

Figure 2: ^{1}H-NMR spectrum of steriod

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