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A NOTE ON THE ANTIINFLAMMATORY ACTIVITY OF BERGENIN

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BERGENIN, a gluco isocoumarin isolated from the flowers of *Peltophorum pterocarpum* Backer ex. K. Heyne (Fam. Leguminosae)[1] has been investigated for antiinflammatory activity by the carrageenin induced rat paw *oedema*[2].

Albino rats of either sex (130–150 g) were used as experimental animals. The animals were given food and water *ad libitum* both before and during the experiment and maintained at room temperature. The test compound suspended in sterile water to give the desired concentration, was administered to three different groups of rats (5 rats in each group) at doses of 60 mg/kg BW, 120 mg/kg BW and 240 mg/kg BW and the reference drug—phenyl butazone was given to a group at a dose of 100 mg/kg BW.

A solution of carrageenin (1% W/V, 0.1 ml) was injected into the sub-planter tissue of one of the hind feet. The paw volume was measured immediately after injecting carrageenin and again after 3 hr by the Plethysmometric method[3]. The results were expressed as increase in foot volume in ml over the initial volume (table 1).

Table 1 *Effect of phenyl butazone and bergenin on carrageenin induced rat paw oedema.*

Treatment	Dose mg/kg BW	Mean increase in paw volume ± S.E. (ml)	% inhibition
Control	—	0.28 ± 0.01	—
Phenyl butazone	100	0.08 ± 0.01	72.6
Bergenin	60	0.16 ± 0.01	44.1
	120	0.13 ± 0.01	53.6
	240	0.09 ± 0.01	65.5

From these results it is clear that bergenin produces a dose dependent inhibition of carrageenin induced rat paw oedema. Its potency is maximum at higher doses, comparable to that of the reference drug viz. phenylbutazone. This test offers a significant predictive value for its clinical usefulness.

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ISOLATION OF PHYTOSTEROLS FROM SUGARCANE PRESS MUD AND MICROBIAL CONVERSION OF THE PHYTOSTEROLS TO 17-KETOSTEROIDS

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THE sugarcane press mud is a waste material of sugar industries. It contains a number of valuable materials like wax and lipids which have commercial potential[1]. It has been suggested that phytosterol in the press mud may serve as a potential source of sterol for production of biologically active steroids[2]. In this communication, the isolation of phytosterols alongwith wax and lipids from press mud and microbial conversion of the phytosterols to androst-4-ene-3,17-dione (AD) and androsta-1,4-diene-3,17-dione (ADD) which can serve as intermediates in the synthesis of steroid hormones are described.

Benzene extraction of the dried sugarcane press mud (obtained from Assam Cooperative Sugar Mills Ltd.,

Dergaon, India) showed 10–12% crude wax on dry press mud basis. Further fractionation of the crude wax into hard wax, soft wax, resin, fatty acids, unsaponifiable matter (USM), higher alcohols/hydrocarbons and phytosterols is shown in the isolation scheme depicted in figure 1. Fatty acids were estimated as methyl esters by gas liquid chromatography (GLC) and consisted of mainly $C_{16\,0}$, $C_{18\,1}$ and $C_{18\,2}$ with some minor acids. Acetone extraction of USM gave cold acetone insoluble fraction which did not contain any sterol as indicated by negative Liebermann-Buchard reaction. Thin layer chromatographic (TLC) analysis of this fraction on silica gel G coated plate using petroleum ether:ethylacetate (12:1) as the developing solvent system showed a single spot in iodine vapour. The fraction had melting point of 80–82°C and IR (KBr pellets) bands occurred at 3350, 2900, 2825, 1474, 1465, 1065 cm^{-1} indicating the presence of alcohol (–OH) functional groups. GLC analysis of the fraction revealed at least 8 peaks. The fraction was tentatively considered to consist of higher fatty alcohols and hydrocarbons. Melting point, IR, and MS of the phytosterol fraction indicated the presence of β-sitosterol, stigmasterol and campesterol. GLC analysis of the purified sterol using authentic sterol samples to identify the peaks following the methods described by Goswami *et al*[3] showed that it contained 18.7% campesterol, 39.6% stigmasterol, 34.0% β-sitosterol with two minor unidentified peaks (1.7 and 6.0%) (figure 2). The yield of purified phytosterol was 0.36–0.43% of dry press mud. The purified phytosterol was used for microbial conversion to 17-ketosteroids.

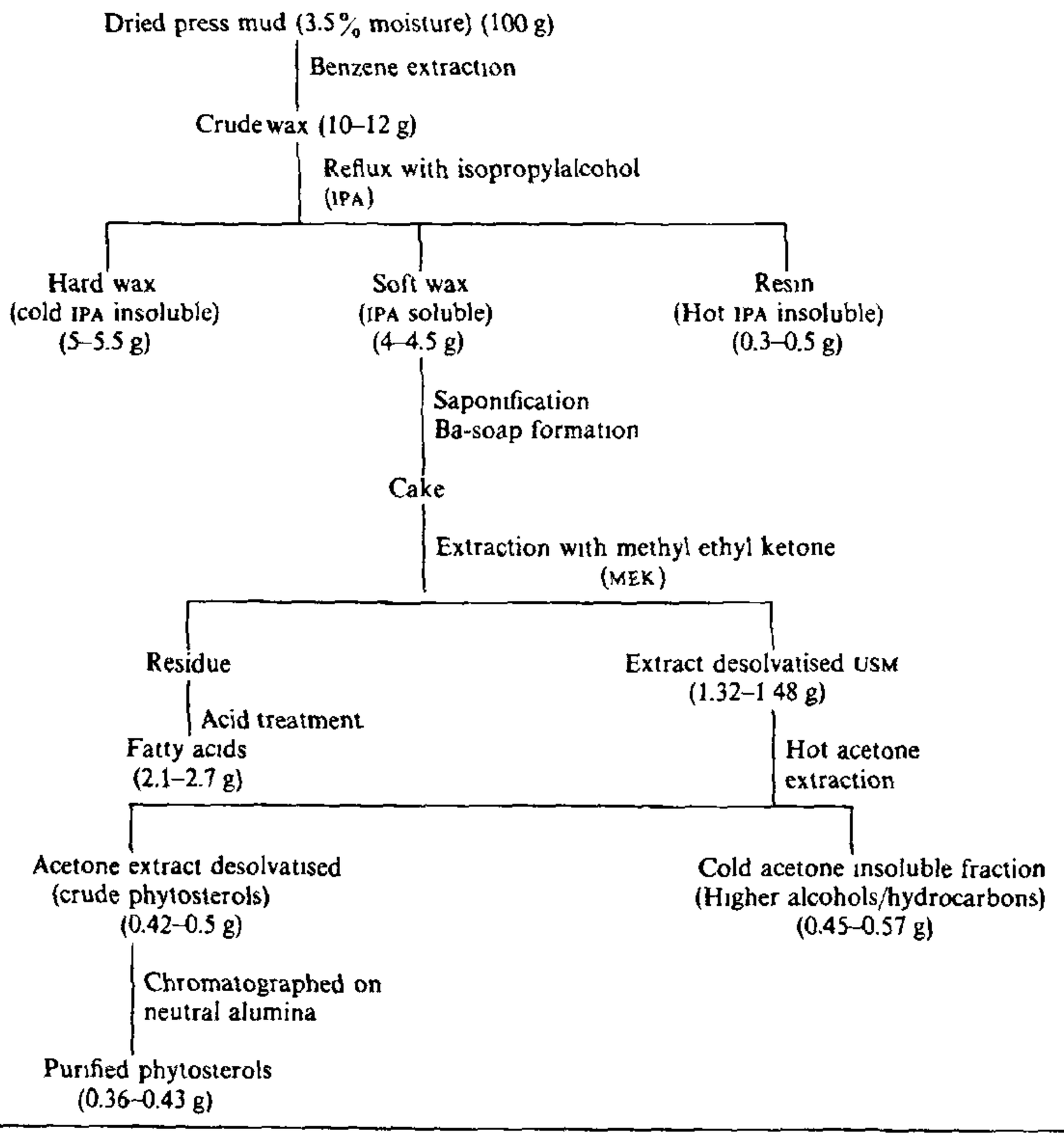

Figure 1. Isolation scheme for wax, lipids and phytosterols from sugarcane press mud.

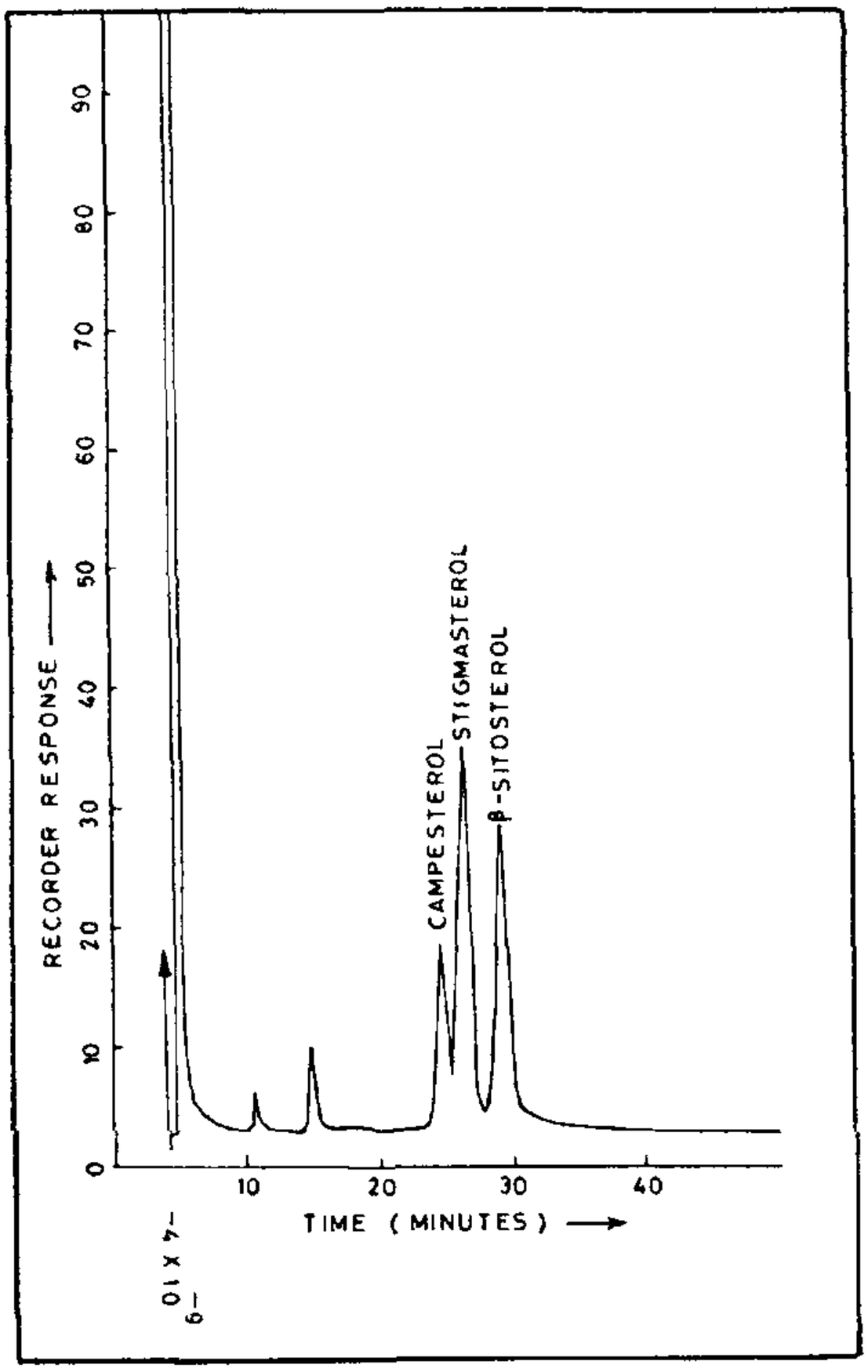

Figure 2. GLC separation of individual sterols from purified sterol mixture obtained from press mud.

Arthrobacter 317 isolated from the soil by enrichment culture technique was used for the study following the fermentation technique described previously[3,4]. The organism consumed more than 70% of phytosterol (5 g/l) in 48 hr of incubation. For 17-ketosteroid production 50 ml of sterile mineral medium homogenized with 0.2% phytosterols was inoculated with 5 ml of inoculum preparation as described elsewhere[3,4]. The flasks were then kept in a rotary shaker (180 rpm) at 30–33°C for 24 hr to build up active biomass. This was then followed by further addition of phytosterol (0.2%) and α,α'-dipyridyl (3.4×10^{-4} M) which inhibits the steroid ring degradation reactions[5]. The incubation was continued for a further 24 hr after which the fermentation broth was filtered and the residue and the filtrate were extracted separately with ethylacetate. After desolvatisation of the extracts under reduced pressure the residue was treated with spectroscopic grade chloroform and analysed for metabolites by GLC[3]. The chromatographic peaks were identified by comparing with the retention times of authentic samples of ADD, AD, Δ^4-stigmasten-3-one, stigmasterol acetone, β-sitosterol, stigmasterol and campesterol.

It was found that 17-ketosteroids constituted 95% of the steroidal compounds present in the aqueous filtrate with undegraded sterols constituting only 1.3%

Four unidentified peaks were observed but these accounted to only 2.86% of the total steroids in the filtrate. The filtration residue containing cell debris and sterol particles showed that undegraded sterols constituted 97.7% of the total steroidal compounds with minor amounts of Δ^4-3-one derivatives of sterols (0.3%) and traces of sterol acetates. Five unidentified peaks were observed but these constituted only 1.7% of total steroids. Undegraded sterols comprised 22.9% campesterol, 38.4% stigmasterol and 36.4% β-sitosterol. The composition of the undegraded sterols indicated that the organism had no strong preference for a particular component of the press mud phytosterols for degradation.

A quantitative analysis using GLC showed that yield of 17-ketosteroids from press mud phytosterols was 20.8% of the phytosterols added. ADD constituted 91% of the 17-ketosteroids formed with AD comprising the rest. These results indicated that press mud phytosterols could serve as the starting material for microbial production of commercially important C_{19}-steroids.

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