SHORT REPORT

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CONSTITUENTS OF TYPHA ELEPHANTINA*

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Abstract

Examination of the fruit of Typha elephantina *Roxb. (Typhaceae) revealed the presence of four constituents : pentacosane, 1-triacontanol, β-sitosterol and β-sitosteryl-3-O-β-D-glucopyranoside.*

Typha Linn., the only genus in the family Typhaceae and commonly known as cattails, is a hardy perennial herb often growing gregariously in fresh water and marshy areas[2-4]. According to Smitinand[5], the only species present in Thailand is *T. elephantina* Roxb. (synonymous with *T. angustifolia* Linn.). This species has been used in traditional Indian medicine as a coolant, an aphrodisiac, and in the treatment of leprosy and the rootstalk has been employed in Eastern Asia for the treatment of dysentery, gonorrhea and measles[2,6]. There have been no previous reports of any phytochemical study on this species and our initial screening indicated that all parts of the plant gave a positive result with the Liebermann-Burchard test. This test indicates the presence of steroidal compounds and as the fruit of the plant had particularly high levels of these components, this source was investigated. This report deals with the extraction, isolation and identification of four constituents from the fruit of *Typha elephantina.*

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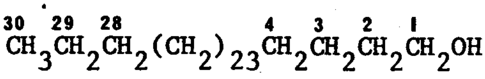
* Part III in the series of "Studies on Thai Medicinal Plants." For Part II see ref. (1).

The residue from ethanol extraction of the fresh fruit was triturated with petroleum ether and resultant filtrate was concentrated and separated into four pure components by column chromatography as described in the Experimental section. The first component to be eluted was a very non-polar low-melting solid, which was shown by several spectroscopic methods to be the linear hydrocarbon pentacosane **1.** The electron impact mass spectrum (MS EI) established that the exact mass was that of a C_{25} saturated alkane and the chemical ionization mass spectrum (MS CI) confirmed this parent peak by the presence of the expected intense fragment for $M\text{-}C_2H_5$[7]. Both spectra gave fragmentation patterns consistent with a linear hydrocarbon and this was confirmed by the ^{13}C-NMR spectrum which exhibited only one methyl resonance at δ 14.1 [C(1) and C(25) are equivalent] in addition to the expected signals for the methylene carbons[8]. Comparison of the natural product with an authentic sample of **1** confirmed they were identical.

The second component eluted was also a solid and the CI mass spectral fragmentation pattern was that expected[7] of a linear C_{30} alcohol, namely 1-triacontanol, **2.** The EI mass spectrum with a strong $M\text{-}H_2O$ fragment also supported this conclusion. The ^{13}C-NMR spectrum, which has not been reported previously, confirmed that the compound was a linear, primary alcohol as it contained only one methyl resonance (δ 14.1) and the expected[8] signals at δ 63.1, 32.9 and 25.8 for C(1) - C(3), respectively. Other data recorded in the Experimental section confirmed that this second constituent was 1-triacontanol, **2.**

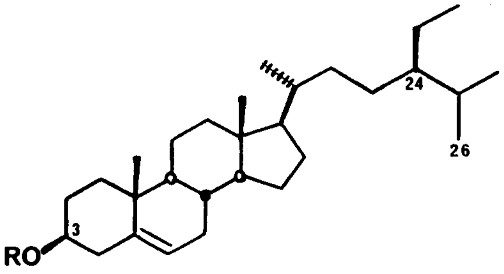
$CH_3(CH_2)_{23}CH_3$

1



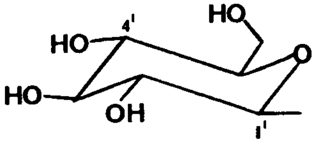
$\overset{30}{C}H_3\overset{29}{C}H_2\overset{28}{C}H_2(CH_2)_{23}\overset{4}{C}H_2\overset{3}{C}H_2\overset{2}{C}H_2\overset{1}{C}H_2OH$

2



3, R = H

4, R =



The third component was crystalline and the color change from pink to blue to green upon treatment with Liebermann-Burchard's reagent suggested it was steroidal in nature. The IR spectrum indicated the presence of hydroxyl and olefin functions and the EI mass spectrum, with a very weak parent ion for $C_{29}H_{50}O$ and an intense M-H_2O peak, was characteristic of Δ^5 -3-β-sterols[9]. Both the ^{1}H- and the ^{13}C-NMR spectra were in accord with those published previously for β-sitosterol[10-12] and thus on the basis of data we have collected we conclude the third constituent is β-sitosterol, **3.**

The fourth and most polar component was a high-melting solid that gave a positive Liebermann-Burchard test, suggesting it was also steroidal in nature. The IR spectrum of the compound exhibited a strong OH absorption and the EI mass spectrum displayed the same base peak (m/z 396) and a similar fragmentation pattern as **3,** implying that this final component was a derivative of β-sitosterol. The ^{13}C-NMR spectrum of the substance displayed the 29 resonances present in β-sitosterol plus six additional downfield signals. Comparisons of the ^{13} C-NMR spectrum of this component with that of the β-glucoside of β-sitosterol[12, 13] suggested they were the same compound. The ^{1}H-NMR data reported previously for this glucoside are very limited[14] so in the Experimental we have recorded a more complete analysis of a spectrum obtained on a 270 MHz spectrometer. All other data obtained for this fourth constituent support the conclusion that it is β-sitosteryl-3-O-β-D-glucopyranoside, **4.**

As *Typha elephantina* has been used for medicinal purposes, we list below some of the biological activities reported for three of the constituents we have shown to be present in the fruit of this plant. 1-Triacontanol (**2**) is known to possess plant growth regulating activity[15] while β-sitosterol (**3**) has been reported to exhibit anti-inflammatory and antipyretic activity[16]. **3** has also been shown to have significant anti-tumor activity against a number of screening systems[17] and its use for the treatment of hypercholesterolemia has been investigated[18]. The β-glucoside **4** has been shown recently to be active against P 388 leukemia[19]. Finally, the presence of both pentacosane (**1**) and β-sitosterol (**3**) have been reported in other *Typha* species[20].

Instrumentation : Melting points were determined in a Gallenkamp apparatus and IR spectra were recorded on a Shimadzu Model IR 440 or a Perkin-Elmer 1330 spectrophotometer. Mass spectra were obtained on a Jeol Model DX 300, a Varian MAT CH7 or a VG Micromass 7070F spectrometer operating at 70 eV. NMR spectra were obtained on a Bruker WH 400 or a Jeol GX 270 spectrometer. A sample of pentacosane was obtained from Sigma Chemical Co.

Plant Material : The fruit of *Typha elephantina* Roxb. was collected from Nakhonpathom province, Thailand in August 1984 and were authenticated by comparison with herbarium specimens in the Botany Section, Technical Division, Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand. A voucher specimen of plant material has been deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

Isolation of **1-4.** The fresh fruit from *T. elephantina* (10 kg) was blended with 95% ethanol, macerated twice over a period of 3 days and then filtered. The filtrate was concentrated under reduced pressure and the resultant residue was triturated with petroleum ether until no trace of steroids could be detected in the extracts. The combined extracts were concentrated to give a greenish residue (18.5 g). The residue was divided into 18 equal portions and each portion was chromatographed on a silica gel column (2.5 × 16 cm). Elution with chloroform gave 30 25-ml fractions and with chloroform : methanol (95 : 5) gave an additional 15 25-ml fractions. Fraction 1 gave component **1** (42 mg, 0.00042%), fractions 13-14 gave **2** (35 mg, 0.00035%), fractions 17-22 gave **3** (180 mg, 0.0018%) and fractions 35-39 gave **4** (92 mg, 0.00092%).

Pentacosane, **1.** M.p. 50.5-52.0°C, mixed m.p. 52-54°C [lit. (21) 53.3°C]; MS EI m/z (rel. int.) 352 (M^+, 6), 323 (3), 309 (5), 295 (5), 281 (6), 267 (8), 253 (9), 239 (10), 225 (11), 211 (14), 197 (18), 183 (17), 169 (20), 155 (37), 141 (39), 127 (47), 113 (73), 99 (100). For comparison of some of the lower molecular mass ions see ref. (22); MS CI (isobutane) m/z 352 (M^+, 18), 351 (67), 323 (100); ^{13}C-NMR (100 MHz $CDCl_3$) δ 14.1 (C-1, C-25), 22.7 (C-2, C-24), 29.4 (C-4, C-22) 29.7 (C-5 to C-21), 32.0 (C-3, C-23).

1-Triacontanol, **2.** M.p. 82-83°C [lit. (21) 86.5°C]; MS EI m/z (rel. int.) 421 (M-OH, 2), 420 ($M-H_2O$, 5), 392 (3), 336 (1), 294 (1), 280 (1), 264 (1), 252 (1), 238 (2), 224 (2), 210 (3), 196 (3), 182 (4), 181 (5), 167 (7), 153 (10), 139 (15), 125 (29), 111 (53), 97 (98), 85 (43), 83 (93), 71 (61), 69 (60), 57 (100); MS CI (isobutane) m/z 439 (M+1, 4), 438 (M^+, 8), 437 (25), 422 (33), 421 (100), 393 (17); ^1H-NMR (400 MHz, $CDCl_3$) δ 3.63 (t, J = 6.0 Hz, 2H), 2.16 (s, 1H), 1.26 (br s, 56H), 0.88 (t, J = 6.6 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 63.1 (C-1), 32.9 (C-2), 32.0 (C-28), 29.7 (C-5 to C-27), 29.4 (C-4), 25.8 (C-3), 22.7 (C-29), 14.1 (C-30).

β-*Sitosterol,* **3.** M.p. (abs. EtOH) 137-140°C [lit. (23) 136-137°C]; TLC [ether : petrol (5 : 95)] R_f 0.27; IR see ref. (23); MS EI m/z (rel. int.) 414 (M^+, 85), 396 (100), 381 (36), 255 (61), 213 (41), 163 (37), 161 (47), 159 (58), 147 (66), 145 (85), 135 (41), 133 (55), 131 (43), 121 (53), 119 (51), 109 (48), 107 (75), 105 (79), 95 (79), 93 (64), 91 (65), 83 (50), 81 (100); ^1H - and ^{13}C-NMR, see ref. 10-12.

β-*Sitosteryl-3-O-*β*-D-glucopyranoside,* **4.** M.p. (chloroform/petrol) 255°C [lit. (24) 257-258°C]; TLC [ether : petrol (2 : 3)] R_f 0.50; IR and MS EI, similar to ref. (24); ^1H-NMR (270 MHz, pyr.- D_5) δ 5.35 (br s, H-6), 5.18 (d, J = 7.6Hz, H-1′), 4.58 (dd, J = 5.3, 11.5Hz, H-6′a), 4.48 (dd, J = 2.3, 11.5, H-6′b), 4.32 (t, J = 8.6Hz, H-3′), 4.29 (t, J = 8.6Hz, H-4′), 4.10 (dd, J = 7.6, 8.6Hz, H-2′), 4.01 (m, H-3 and H-5′), 1.01 (s, Me-19), 0.91 (d, J = 7.6Hz, Me-21), 0.88 (d, J = 6.0Hz, Me-26), 0.86 (t, J = 7.2Hz, Me-29), 0.68 (s, Me-18); ^{13}C-NMR similar to ref. 12,13.

Acknowledgements

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บทคัดย่อ

จากการตรวจสอบของต้นกกช้าง *Typha elephantina* วงศ์ Typhaceae พบสารประกอบ 4 ชนิด คือ pentacosane, 1-triacontanol, β-sitosterol และ β-sitosteryl-3-O-β-D- glucopyranoside