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CHEMICAL COMPONENTS OF *Leptopus chinensis*

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Six substances were isolated from the branches and leaves of the Chinese herb Leptopus Chinensis *(Bunge) Pojark. by column chromatography for the first time. Their structures were elucidated as 3α-hydroxy-friedelan-2-one, saccharose, triacontanol, friedelane-2α,3β-diols, β-sitosterol-3-O-β-D-glucoside, and β-sitosterol on the basis of x-ray, chemical, and spectroscopic methods.*

Key words: *Leptopus chinensis* (Bunge) Pojark., triacontanol, 3α-hydroxyfriedelan-2-one; saccharose; friedelane-2α,3β-diols, β-sitosterol, β-sitosterol-3-O-β-D-glucoside, oriental medicine.

Leptopus chinensis (Bunge) Pojark., a herb of the Euphorbiaceae family, is widely distributed in China, namely Hubei, Hunan, Guangxi, Sichuan, and Yunnan. Its branches and leaves have long been known as a herbal medicine by local people to treat several kinds of disease, such as abdominal pain, malnutrition due to parasitic infestation, and even paralysis [1, 2]. Initial pharmacological experiments showed a degree of inhibition to the growth of cancer cells. A number of descriptions on the medical uses of this plant can be found in many Chinese books and dictionaries, but studies of the structure of the main constituents of this plant have not yet been reported. It is necessary to start the study on the chemical components of this oriental medicine. This first report describes the isolation from *Leptopus chinensis* of triacontanol, 3α-hydroxyfriedelan-2-one, saccharose, friedelane-2α,3β-diols, β-sitosterol, and β-sitosterol-3-O-β-D-glucoside and the structure elucidation of these crystalline substances by IR, MS, NMR, and x-ray analysis. Other components of this plant will be presented in subsequent reports.

The branches and leaves of *Leptopus chinensis* were extracted with 95% methanol and the extract was treated as described in the Experimental section. The crude materials were separated by chromatography using silica gel.

Compound 1: $C_{30}H_{62}O$. This crystalline substance was identified by mass spectrometry to be 1-triacontanol [$CH_3(CH_2)_{28}CH_2OH$]. The mass spectrum was typical of long-chain alcohols. Although the parent peak (M^+) was not observed, there was a large peak at *m/z* 420 that resulted from loss of water (M^+-18), and a rearrangement ion at *m/z* 329 that resulted from the combined loss of ethylene and water. The series of alkyl and alkene ions showed no evidence of branching. The structure was confirmed by ^{1}H NMR. The 3H signal at δ 0.885 was assigned to CH_3, and 1.254–1.584 and 3.641 to -($C\underline{H}_2$)$_{28}$- and $C\underline{H}_2$-OH respectively.

Compound 2: $C_{30}H_{50}O_2$. Its molecular formula was determined by MS and elemental analysis. A positive Lieberman-Burchardt test was obtained.

This rodlike crystal was identified by NMR as 3α-hydroxyfriedelan-2-one with physical constants and spectral data in agreement with the published values [4, 5]. The ^{1}H NMR and ^{13}C NMR spectral data were almost identical with 3α-hydroxyfriedelan-2-one [4, 5] (see Experimental section).The structure was further confirmed by x-ray crystallography.

Compound 3 was identified as saccharose by comparing its IR with that of authentic saccharose.

Compound 4. Colorless needles, mp 283–284.5°C, $C_{30}H_{52}O_2$, determined by EIMS. A positive Lieberman-Burchardt test was obtained. This crystal was identified by NMR as friedelane-2α,3β-diols, with physical constants and spectral data in agreement with [6, 7] (see Experimental section). The structure was further confirmed through its DEPT data.

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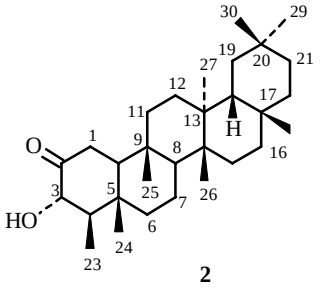

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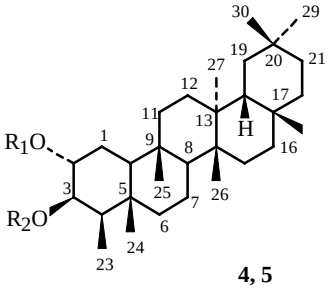

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4: $R_1 = R_2 = H$
5: $R_1 = R_2 = COCH_3$

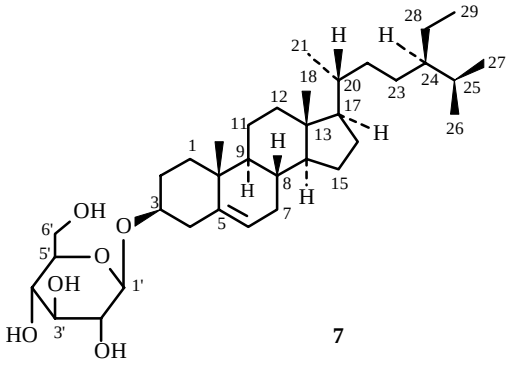
Compound 5, colorless needles, mp 235–236°C, $C_{34}H_{56}O_4$, synthesized by the acetylating reaction between compound **4** and acetic anhydride.

^{1}H NMR ($CDCl_3$, δ, ppm): the double signals of 0.801, 0.819 were signals of 23-CH_3, and 0.838, 0.910, 0.942, 0.992, 0.992, 1.035, 1.171 were signals of the seven other methyl groups. The presence of two acetoxyl groups was deduced by ^{13}C NMR (δ 169.991, 169.680). This crystal was identified with physical constants and spectral data in agreement with the published values [6–8] (see Experimental section). The structure of compound **4** was further confirmed by the spectral data of compound **5**.

Compound 6, white powder, $C_{29}H_{50}O$, mp 134–135°C.

^{1}H NMR ($CDCl_3$, δ, ppm): 5.353 (1H, C_6-H), 3.528 (1H, m, 3α-H). This powder was identified with spectral data in agreement with the published values [9]. Compound **6** was confirmed as β-sitosterol.

Compound 7, white powder, $C_{35}H_{60}O_6$, mp 290°C, a positive Lieberman-Burchardt test was observed. Although the parent peak (M^+) was not observed, there was a peak at *m/z* 414 that resulted from loss of the glucose side. By referencing some of the published spectral data [9], the structure of compound **7** was finally determined by the spectral data of its DEPT, ^{1}H–^{1}H COSY, and ^{13}C–^{1}H COSY as β-sitosterol-3-O-β-D-glucoside.



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EXPERIMENTAL

Plant Material. *Leptopus chinensis* (Bunge) Pojark. plant material was provided by the Ruzhou Foreign Trade Bureau, Ruzhou City, Henan Province and was taxonomically identified at the Pharmaceutical Department, Henan Medical University.

Extraction of Triacontanol (Compound 1). Approximately 5 kg branches and leaves of *Leptopus chinensis* (Bunge) Pojark. were ground and then soaked in 95% EtOH for two weeks. The mixture was filtered and a green solution was obtained. The solution was concentrated under reduced pressure at 40–70°C. The concentrated solution was first extracted by ethyl ether and then by ethyl acetate. The ethyl acetate solution was dried and evaporated under reduced pressure. The ethyl acetate extract was further separated by column chromatography using $CHCl_3$–$CH_3COOC_2H_5$ (5:1). The rich fractions were collected and combined, and the solvent was evaporated. The extract was further purified using $CHCl_3$–$CH_3(CH_2)_4CH_3$–$CH_3COOC_2H_5$ (10:10:1) to elute the column. White crystals were observed in conical flask 4. After filtration, white plate crystals of compound **1** were obtained.

Compound 1: $C_{30}H_{62}O$, mp 85°C, IR ν_{max} cm^{-1}: 3430 (OH), 2920, 2850. EIMS *m/z*: 420 (M^+-18), 392, 378, 364, 349, 334, 320, 306, 292, 278, 264, 237, 222, 209, 195, 181, 167, 153, 139, 125, 111, 97, 83, 69, 57, 43.

^{1}H NMR ($CDCl_3$, δ, ppm): 0.885 (3H, t, -C<u>H</u>$_3$), 1.254–1.584 (54H, m, CH_3-(C<u>H</u>$_2$)$_{27}$-), 3.641 (2H, t, -C<u>H</u>$_2$-OH).

^{13}C NMR ($CDCl_3$, δ, ppm): 63.135 (C-1), 32.873 (C-2), 31.946 (C-3), 29.717–29.386 (C-4–C-27), 25.757 (C-28), 22.712 (C-29), 14.139 (C-30).

Extraction of 3α-Hydroxyfriedelan-2-one (Compound 2) and Saccharose (Compound 3). Approximately 5 kg branches and leaves of *Leptopus chinensis* (Bunge) Pojark. were ground and then soaked in 95% EtOH for two weeks. The extract was concentrated under reduced pressure and was successively partitioned with petroleum-ether, ethyl acetate, acetone, and ethanol. The petroleum-ether fraction was subjected to column chromatograghy and eluted with petroleum-ether–ethyl acetate (10:1). The fraction between conical flask 38 and 42 was collected and combined, and the eluting solvent was evaporated. The crude residue was subjected to repeated column chromatography on silica gel and was eluted with *n*-hexane–ethyl acetate (4:1) to yield rodlike crystals.

Compound 2: $C_{30}H_{50}O_2$, mp 232–234°C. EIMS *m/z*: 443 $(M+H)^+$.

^{1}H NMR ($CDCl_3$, δ, ppm, J/Hz): 2.541 (1α-H), 2.397 (1β-H), 3.822 (dd, J = 11.6, 3β-H), 1.840 (6β-H), 1.048 (CH_3-23), 1.034 (CH_3-24), 0.891 (CH_3-25), 1.009 (CH_3-26), 0.979 (CH_3-27), 1.173 (CH_3-28), 0.993 (CH_3-29), 0.943 (CH_3-30).

^{13}C NMR ($CDCl_3$, δ, ppm): 36.10 (C-1), 211.69 (C-2), 76.98 (C-3), 54.60 (C-4), 38.10 (C-5), 40.72 (C-6), 17.64 (C-7), 53.18 (C-8), 37.60 (C-9), 60.47 (C-10), 35.10 (C-11), 30.37 (C-12), 39.60 (C-13), 38.40 (C-14), 32.39 (C-15), 36.00 (C-16), 30.03 (C-17), 42.85 (C-18), 35.36 (C-19), 28.18 (C-20), 32.80 (C-21), 39.26 (C-22), 10.79 (C-23), 14.18 (C-24), 17.50 (C-25), 20.117 (C-26), 18.55 (C-27), 32.14 (C-28), 31.80 (C-29), 35.03 (C-30). After two days a large quantity of saccharose-like crystals (compound **3**) appeared in the ethanol fraction. The identity of compound **3** as saccharose was confirmed using IR with saccharose as a standard.

Extraction of Friedelane-2α,3β-diols (Compound 4) and Synthesis of Compound 5. The petroleum-ether fraction was subjected to column chromatograghy and gradient elution separation with petroleum-ether–ethyl acetate. The fraction between conical flask 142 to 190 was collected with petroleum-ether–ethyl acetate (3:1) and the eluting solvent was combined and evaporated. The crude residue was subjected to repeated column chromatography on silica gel to yield needlelike crystals (compound **4**, $C_{30}H_{50}O_2$, mp 232–234°C. EIMS *m/z*: 444 (M^+), 429, 291, 273, 249, 222, 191, 181, 163, 135, 123, 109, 81, 69 (base peak), 55, 41. IR (ν^{KBr}, cm^{-1}): 3460 (br OH), 2924, 1520, 1380, 1350, 1050,1040, 1020.

^{1}H NMR ($CDCl_3$, δ, ppm): 3.544 (2-H, m), 3.984 (3-H, m).

^{13}C NMR ($CDCl_3$, δ, ppm): 23.938 (C-1), 71.399 (C-2), 76.512 (C-3), 43.680 (C-4), 37.792 (C-5), 41.369 (C-6), 17.516 (C-7), 52.276 (C-8), 36.518 (C-9), 53.235 (C-10), 35.494 (C-11), 30.610 (C-12), 39.704 (C-13), 38.394 (C-14), 32.327 (C-15), 36.061 (C-16), 30.025 (C-17), 42.829 (C-18), 35.348 (C-19), 28.180 (C-20), 32.810 (C-21), 39.288 (C-22), 10.904 (C-23), 15.914 (C-24), 18.160 (C-25), 18.742 (C-26), 20.142 (C-27), 32.125 (C-28), 31.788 (C-29), 35.048 (C-30).When compound **4** was added dropwise to a solution of pyridine and acetic anhydride with stirring, white fine needle crystals (compound **5**) were obtained after the solution was evaporated.

Compound 5: mp 235–236°C, $C_{34}H_{56}O_4$

^{1}H NMR ($CDCl_3$, δ, ppm): 0.801 (0.819) (23-CH_3, d); 0.838, 0.910, 0.942, 0.992, 0.992, 1.035, 1.171, 4.793 (H-2, m), 4.868 (H-3, m).

^{13}C NMR (δ, $CDCl_3$): 21.686 (C-1), 70.861 (C-2), 73.917 (C-3), 43.795 (C-4), 37.497 (C-5), 41.294 (C-6), 17.637 (C-7), 52.978 (C-8), 36.454 (C-9), 53.116 (C-10), 35.390 (C-11), 30.377 (C-12), 39.660 (C-13), 38.403 (C-14), 32.293 (C-15), 36.080 (C-16), 30.037 (C-17), 42.861 (C-18), 35.314 (C-19), 28.165 (C-20), 32.851 (C-21), 39.257 (C-22), 10.488 (C-23), 15.035 (C-24), 18.015 (C-25), 18.522 (C-26), 20.044 (C-27), 32.118 (C-28), 31.855 (C-29), 34.962 (C-30), 169.991 (-$COCH_3$), 169.680 (-$COCH_3$).

Extraction of β-Sitosterol (Compound 6). The petroleum-ether fraction was subjected to column chromatograghy and gradient elution separation with hexane–petroleum-ether (8:1, 6:1, 4:1, 2:1). The fraction between conical flask 66 to 73 was collected and the eluting solvent was combined and evaporated to yield a white powder (compound **6**). $C_{29}H_{50}O$, mp 134–135°C, EIMS *m/z*: 414(M^+) (base peak), 412, 396, 382, 351, 329, 273, 255, 213, 163, 159, 145, 121, 119, 105, 95, 81, 43.

^{1}H NMR (δ, ppm, $CDCl_3$): 5.353 (1H, C_6-H), 3.528 (1H, m, 3α-H).

Extraction of β-Sitosterol-3-O-β-D-glucoside (Compound 7). The ethyl acetate fraction was subjected to column chromatograghy with chloroform–ethyl acetate–methanol (5:4:1). The fraction between conical flask 45 to 53 was collected and the eluting solvent was combined and evaporated to yield a white powder. The product was purified by re-chromatography

(compound **7**). $C_{35}H_{60}O_6$, mp 290°C, EIMS *m/z*: 414, 396 (base peak), 382, 354, 298, 285, 275, 255, 229, 213, 199, 187, 173, 159, 145, 129, 119, 105, 81, 69, 57.

^{1}H NMR (δ, ppm, DMSO, $CDCl_3$): 5.331 (1H, d).

^{13}C NMR (δ, DMSO, $CDCl_3$): 37.020 (C-1), 33.525 (C-2), 77.069 (C-3), 39.757 (C-4), 140.631 (C-5), 121.416 (C-6), 29.456 (C-7), 31.595 (C-8), 49.783 (C-9), 36.406 (C-10), 20.787 (C-11), 38.489 (C-12), 42.044 (C-13), 56.360 (C-14), 24.060 (C-15), 27.991 (C-16), 55.608 (C-17), 11.873 (C-18), 19.298 (C-19), 35.677 (C-20), 19.125 (C-21), 31.595 (C-22), 25.599 (C-23), 45.316 (C-24), 28.875 (C-25), 19.918 (C-26), 18.810 (C-27), 22.786 (C-28), 11.978 (C-29), 100.957 (C-1′) 73.655 (C-2′), 76.050 (C-3′), 70.278 (C-4′), 77.069 (C-5′), 61.276 (C-6′).

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