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# ALKALOIDS AND OTHER COMPOUNDS OF *SYMPHYTUM TUBEROSUM*

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**Key Word Index**—*Symphytum tuberosum*; Boraginaceae; anadoline; echimidine; strophanthobiose; sugars; amino acids; steroidal alcohols; hydrocarbons.

## INTRODUCTION

The presence of senecio alkaloids in the Boraginaceae has been known for some time, Culvenor [1–4] and Men'shikov [5–8] obtained a number of new senecio alkaloids from plants of this family. However, there are only a few studies with *Symphytum* species. Echimidine and symphytine [9] were isolated from *S. officinalis*. Echimidine, symphytine, an unknown alkaloid $C_{20}H_{27-29}O_6N$ and a new alkaloid, anadoline were isolated from *S. orientale* [10–12]. There is only one paper dealing with *S. tuberosum* [13], some sugars and amino acids together with allantoin being reported.

In this study the light petrol and chloroform extracts of the whole plant yielded *n*-heneicosane, palmitone, tricosanol as well as sitosterol and another steroidal alcohol $C_{28}H_{50}O$. The alcoholic extract showed the presence of mannose, glucose, galactose and strophanthobiose which was found earlier as a combined sugar in *Strophanthus kombé* [14], this is the first time it has been isolated in the free state. The alcoholic extract also yielded six alkaloids, two of them identified as anadoline and echimidine. In the aqueous extract of the plant the following amino acids were detected: aspartic acid, glycine, leucine, serine, valine, alanine, glutamic acid, proline, methionine, isoleucine, phenylalanine, histidine and lysine (cf. also ref. [13]). Although allantoin is common in *Symphytum* species and has been reported in *S. tuberosum* [13], we could not detect it in our plant material.

## EXPERIMENTAL

Mp's were not corrected; IR spectra were recorded in KBr; NMR were taken in $CDCl_3$ using TMS as internal standard at 60 MHz. MS taken in a double focus 6e instrument. Known compounds were identified by TLC, mmp, IR, and NMR comparison. The plant was collected from Belgrat forest near Istanbul, identified by Prof. Dr. A. Baytop (voucher specimen ISTE 17761).

*Extraction and isolation of the compounds.* After drying and powdering the whole plant was extracted with light petrol, $CHCl_3$, EtOH and $H_2O$, respectively. After TLC checking, light petrol and $CHCl_3$ extracts were combined and chromatographed on alumina (activity III). *n*-Heneicosane, mp 40–40.5°; palmitone, mp 82–83°, IR$\nu_{max}$ $cm^{-1}$:1720; tricosanol, mp 76°, IR$\nu_{max}$ $cm^{-1}$:3460; sitosterol, mp 137°, IR$\nu_{max}$ $cm^{-1}$: 3450, 1480, 1375; 4α-mehylcholestane type steroidal alcohol, mp 122–125°, (Found: C, 83.75; H, 12.27Calcd for $C_{28}H_{50}O$:C, 83.58; H, 12.43%), IR$\nu_{max}$ $cm^{-1}$:3480, 1460, 1385. A monoacetyl derivative formed on acetylation, mp 119–122°, NMR of the acetyl $\delta$ 0.7 (3H, *d*, C-20 Me), 1.00 (3H, *d*, C–4 Me), 2.01 (3H, *s*, acetyl), no vinylic proton. The compound could be a saturated derivative of 4α-methylcholestane. After separation of the crude alkaloids through acid-base extraction, the alcoholic extract was chromatographed on Si gel. Mannose, mp 132°; glucose, mp 144°; galactose, mp 165°, TLC and PC comparison with the standards. Strophanthobiose, mp 205–207°, (Found: C, 47.95; H, 7.60. Calcd for $C_{13}H_{27}O_9$:C, 48.14; H, 7.57%), acetyl derivative, mp 160–162°, acid hydrolysis yielded glucose and cymarose (TLC and PC comparison). NMR of the acetyl derivative $\delta$, 1.00 (3H, d, Me of cymarose), 1.99, 2.01, 2.05 and 2.17 (acetyl singlets for 5 acetyl groups), 4.85, 5.00, 5.20, 5.35, 5.50 and 5.72 other protons as doublets, triplets and multiplets, MS: *m/e* 145 (cymarose), 116 (145–OMe + 2H), 161 ($C_6H_{11}O_5$–2H, glucose), $[\alpha]_D$ + 29 ($H_2O$), lit. value $[\alpha]_D$ + 31 ($H_2O$) [15].

*Separation and identification of alkaloids.* Column separation of the alkaloids was not effective, preparative Si gel G TLC plates in MeOH–$H_2O$ (85:15) were used, 2 main bands were extracted with $CHCl_3$. Echimidine; mp of echimidine picrate 142°, (Found: C, 60.56; H, 7.85; N, 3.47. Calcd for $C_{20}H_{31}O_7N$: C, 60.45; H, 7.80; N, 3.52), IR$\nu_{max}$ $cm^{-1}$: 3350 (OH), 1738, 1700 (ester carbonyls), 1380 (isopropyl). NMR$\delta$, 0.9 (3H, *d*, $J$ = 6.5 Hz), 0.93 (3H, *d*, $J$ = 6.5 Hz), 1.27 (3H, *d*, $J$ = 7 Hz), 1.95 (6H, *s*, 2 × Me). Anadoline, mp 186°, (Found: C, 60.40; H, 7.68; N, 3.48; Calcd for $C_{20}H_{31}O_7N$: C, 60.45; H, 7.80; N, 3.52), IR$\nu_{max}$ $cm^{-1}$:3450 (OH), 1740, 1700 (ester carbonyls), 1650 (double bond), 1387 (isopropyl). NMR$\delta$, 0.9 (3H, *d*, $J$ = 7 Hz), 1.07 (3H, *d*, $J$ = 7 Hz) (both isopropyl methyls), 1.3 (3H, *d*, $J$ = 6.5 Hz, CH–$\underline{CH_3}$), 1.90 (6H, *s*, slight division at the peak, $\underline{CH_3}CH{=}C\underline{CH_3}$), 4.8 (2H, *s*, 2 × OH), 6.8 (1H, *m*, vinylic H), 5.92 (1H, *s*, C–2H), 4.02 and 4.20 (1H each, *dd*, $J$ = 9 Hz, C–3 protons), 2.10 and 2.40 (1H each, *m*, C–5 protons), 4.7 (1H, *br s*, C–8H).

*Identification of amino acids.* Aq. extract of *S. tuberosum* was analysed using a Beckman Multichrom B liquid column chromatography 4255 analyser.

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# ALKALOIDS OF *CALTHA LEPTOSEPALA* AND *CALTHA BIFLORA*

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**Key Word Index**—*Caltha leptosepala*; *Caltha biflora*; senecionine; magnoflorine; *N,N*-dimethyl lindcarpine.

The genus *Caltha* has world-wide distribution in the northern hemisphere and has been the subject of early European reports of toxicity in cattle and horses [1]. In our present investigation of *Caltha leptosepala* and *C. biflora*, we isolated the pyrrolizidine alkaloid senecionine. One of the symptoms on ingestion of this alkaloid (severe gastrointestinal irritation [2]) corresponds to the reported symptom of *Caltha* poisoning [3]. The second alkaloid isolated has PMR, UV, and $R_f$'s (cf ref. 4) identical to those of the quaternary aporphine alkaloid *N,N*-dimethyl lindcarpine. It has now come to our attention that the spectral and physical properties of *N,N*-dimethyl lindcarpine and its isomer, magnoflorine, are being reinvestigated [5]. Since the properties of these two aporphine alkaloids are very similar [6], the aporphine alkaloid could be either of these alkaloids or a mixture of the two. This is the first report to our knowledge of a pyrrolizidine alkaloid occurring in Ranunculaceae and the first report of the co-occurrence of pyrrolizidine and aporphine alkaloids.

## EXPERIMENTAL

*Extraction and isolation.* Air-dried root and aerial parts of *Caltha leptosepala* DC were collected in Larimer County, Cameron Pass, Roosevelt National Forest, Colorado, U.S.A. Air-dried aerial parts of *C. biflora* DC were collected at Hood River Meadows, Mt. Hood National Forest, Oregon, U.S.A. (Specimens deposited in Colorado State herbarium.) *C. leptosepala* dried aerial parts (1 kg) and roots (1 kg), resp, were extracted with $C_6H_6$–BuOH (1:1) soln (6 l.) and 10% $NaHCO_3$ (1.5 l.) for 24 hr. The filtrate was extracted with M $H_2SO_4$ and this aq. soln was then extracted sequentially with $CHCl_3$ at pH 1 and 8.5. The latter $CHCl_3$ extract was chromatographed on Sephadex LH-20 $CHCl_3$–MeOH (1:1). The eluate yielded senecionine as shown by identical UV, IR, PMR, MS and $[\alpha]_D^{25}$ to lit [7–9] data, aerial parts (0.005%) and roots (0.002%). Several minor alkaloids were also detected but not identified. *C. leptosepala* aerial parts were then re-extracted with MeOH and this soln was filtered and evaporated. Residue was treated with 1% $H_2SO_4$ which was then made basic with NaOH and extracted with $H_2O$ satd *n*-BuOH. The *n*-BuOH extract was chromatographed on a low pressure liquid system using a cellulose column and elution with 0.1 M HCl at 7 kg/sq cm, 15 ml/min. The eluate yielded a quaternary aporphine alkaloid which by lit. values [4] is identical to *N,N*-dimethyl lindcarpine. (0.01%): PMR (DMSO-$d_6$) 2.93 (s, 3H, N—Me), 3.40 (s, 3H, N—Me), 3.82 and 3.85 (*d*, 6H, —Me), 6.98 (s, 3H, Ar H's); UV $\lambda_{max}^{MeOH}$ 225 nm, 277 and 320, $\lambda_{max}^{0.01\ N\ HCl\ in\ MeOH}$ 223 nm, 267 and 303. TLC of the extracted roots showed that they also contain this alkaloid. A standard sample was unavailable, and we have been informed that conclusions regarding the identity of any isolated alkaloids as *N,N*-dimethyl lindcarpine or magnoflorine cannot be established at this time [5]. *C. biflora* (1 kg) was treated in a similar manner as *C. leptosepala* but yielded only senecionine (0.001%).

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