

ALKALOIDS FROM *Senecio cineraria* C. D.*A. KLÁSEK^a, V. A. MNATSAKANYAN^b and F. ŠANTAVÝ^a^a *Chemical Institute, Medical Faculty,
Palacký University, 775 15 Olomouc and*^b *Institute of Fine Organic Chemistry, Academy of Sciences Arm.SSR, Yerevan, USSR*

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From the plant *Senecio cineraria* D.C. there was isolated retrorsine (*V*) in addition to the already earlier found jacobine (*I*), senecionine (*II*), and seneciphylline (*III*). The presence of othosenine (*IV*) which, according to the literature, had also been found in this plant, could not be demonstrated.

Within the scope of a systematic investigation of pyrrolizidine alkaloids, carried out at our Institute, we have isolated the alkaloids contained in *Senecio cineraria* D. C. (syn. *S. maritima* L., *S. acanthifolius* HORT.). This plant has already been studied several times for its content of alkaloids. Some authors¹⁻⁴ isolated from it the alkaloids jacobine (*I*), senecionine (*II*) and seneciphylline (*III*) but the content of alkaloids found by them in the collected plant material differs considerably in the individual papers. A short time back, a paper⁵ was published reporting the isolation of othosenine (*IV*) in addition to that of the three above-mentioned alkaloids from *S. cineraria*.

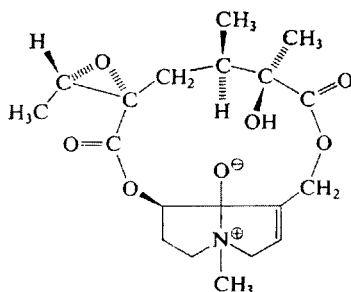
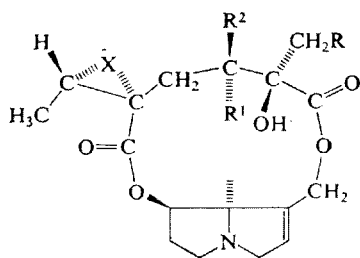
While studying the effect of drying on the content of alkaloids, we have now extracted some of this plant material directly in the fresh state, and some of it after drying and storing for a period of seven months. In these two batches, the content of the mixture of crude alkaloids has differed considerably (0.246 and 0.114% per dry weight). This difference was, however, caused by the presence of a larger quantity of ballast substances in the portion obtained after extraction of the fresh plant because the quantity of the purified mixture of alkaloids was in both portions practically the same (0.093 and 0.090% per dry weight).

Thin-layer chromatography of the crude mixture of alkaloids from the portion obtained by extraction of the fresh plant produced three spots of hR_F 57, 51 and 26. The substance of hR_F 26 was present only in traces. After purification and conversion to hydrochlorides, the mixture gave the crystalline hydrochloride of an alkaloid of hR_F 51 which, after conversion to the free base, was identified as jacobine (*I*).

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After conversion of the mother liquors to free bases, a crystalline substance of hR_F 57 was obtained which, however, according to the $^1\text{H-NMR}$ spectra, was a mixture of senecionine (II) and seneciophylline (III). These two substances were obtained in pure state by chromatography on cellulose. The alkaloid of hR_F 26 could not be isolated as yet because the quantity was too small but, as shown *vide infra*, this alkaloid was retrorsine (V).

After purification, the crude mixture of alkaloids obtained from the dried portion of the plant material yielded a crystalline mixture of substances of hR_F 57, 51 and 26 in the ratio of *c.* 1 : 1 : 1. Column chromatography on Al_2O_3 of the mixture gave a pure substance of hR_F 26 which was identified as retrorsine (V) — this alkaloid was isolated from *S. cineraria* for the first time. The other substances from the chromatography were obtained in a mixture. After conversion to hydrochlorides, the pure jacobine (I) hydrochloride was obtained. The mother liquors were converted to free bases. On crystallization they gave a substance of hR_F 57 which, on the basis of the $^1\text{H-NMR}$ spectra, was identified as a mixture of senecionine (II) and seneciophylline (III).



- I, $R = R^1 = \text{H}$; $R^2 = \text{CH}_3$; $X = \text{O}$
 II, $R = R^1 = \text{H}$; $R^2 = \text{CH}_3$; $X = \Delta^{5,6}$
 III, $R = \text{H}$; $R^1 + R^2 = \text{CH}_2$; $X = \Delta^{5,6}$
 V, $R = \text{OH}$; $R^1 = \text{H}$; $R^2 = \text{CH}_3$; $X = \Delta^{5,6}$

Since in all the portions (even in the alkaloids from N-oxides), thin-layer chromatography did not reveal any other than the four above-mentioned alkaloids, these mixtures were not separated further. Even though on isolation of the alkaloids from *S. cineraria* there could not be demonstrated any longer lasting effect of drying on the quantity of alkaloids, we must take into consideration the effect on the ratio of the alkaloids in the mixture. Thin-layer chromatography of the two portions showed that the qualitative composition of the mixtures of alkaloids in both of them was the same but they differed from each other by the quantitative representation of the

alkaloid. In the portion obtained from the fresh material, the trace amount of retrorsine (*V*) was markedly lower than that obtained from the dry plant. Obviously some alkaloid from the mixture is transformed into retrorsine (*V*) on drying. The composition of the mixtures of alkaloids originally present in the plant as N-oxides was qualitatively the same in the portion obtained from the fresh plant and in that from the dry plant. In both portions, retrorsine (*V*) was, however, present only in traces. We did not succeed to demonstrate the presence of othosenine (*IV*) which, according to the literature, was isolated⁵ from this plant. The chromatographic properties and the melting points of othosenine (*IV*) and retrorsine (*V*) were much alike. In the paper⁵ a spectroscopic study of the identity of the isolated alkaloid with othosenine (*IV*) was not carried out.

EXPERIMENTAL

The melting points have been determined on the Kofler block and are uncorrected. The IR spectra were measured on an Infracan (Hilger), the ¹H-NMR spectra on a Varian T-60 in deuteriochloroform using tetramethylsilane as internal standard. Thin-layer chromatography on silica gel G was carried out using the solvent system benzene-ethyl acetate-diethylamine (7 : 2 : 1); the *R_F* values are expressed as *hR_F* (*R_F* · 100). Spots were detected by spraying with Dragendorff reagent. The solutions of all the substances in organic solvents were dried over anhydrous sodium sulphate.

The plant with the roots was collected in October 1973 in the parcs of Olomouc. Some quantity of the fresh plant was worked up immediately (portion *A*), the rest was dried (portion *B*).

Isolation of Alkaloids from the Portion *A*

The fresh plant (20 kg) was crushed and extracted with 6.60 l of methanol. At the same time, a small sample was used to determine the dry content (23.7%). The methanolic extract was concentrated *in vacuo* to a volume of 3 l, diluted with water 1 : 1, acidified with citric acid, and washed with light petroleum. The aqueous layer was then extracted with 4.400 ml of ether; evaporation yielded 3.68 g of acid and neutral substances which were not worked up further. The aqueous solution was made alkaline with ammonia to pH 10.5 and extracted with 5.600 ml of chloroform. After drying and evaporation, the yield gave 11.7 g of a crude mixture of alkaloids (0.246% per dry weight). The aqueous layer was acidified with hydrochloric acid to Kongo red, 100 g of zinc powder were added and left standing overnight. After filtration and alkalization with ammonia, the solution was extracted with 5.500 ml of chloroform. Drying and evaporation of the extract gave 1.5 g of a crude mixture of alkaloids from N-oxides (0.032% per dry weight). On thin-layer chromatography, the crude mixture of alkaloids produced three spots of *hR_F* 57, 51 (main portion), and 26 (traces). The mixture of alkaloids from N-oxides showed the same qualitative composition, the main portion was, however, formed by the alkaloids of *hR_F* 57.

The crude mixture of alkaloids was dissolved in 5% sulphuric acid, the solution was filtered, washed with ether, made alkaline with ammonia, and extracted with ether (1.61 g of residue) and with chloroform (2.8 g of residue). The total content of the purified mixture of alkaloids was 0.093% per dry weight. The residue after extraction with ether (1.61 g) was dissolved in ethanol and, after addition of ethanolic hydrochloric acid, crystalline hydrochloride (170 mg) of a substance of *hR_F* 51, m.p. 195–197°C was obtained. An attempt to separate the mother liquors

by chromatography on alumina proved to be a failure. The residue after extraction with chloroform (2.8 g) crystallized from ethyl acetate. Thin-layer chromatography showed that the composition of the crystals was, however, the same as that of the residue. On addition of ethanolic hydrochloric acid, the mixture yielded 1.04 g of pure hydrochloride of a substance of hR_F 51, m.p. 196–198°C after recrystallization from ethanol. After conversion to a free base, crystals of m.p. 225–227°C (ethyl acetate), $[\alpha]_D^{24} -44^\circ \pm 2^\circ$ (c 0.46 in chloroform) were obtained. Jacobine (*I*) was reported⁶ to have m.p. 228°C, $[\alpha]_D -46^\circ$ (chloroform). On the basis of the mixed melting point, the IR and the ¹H-NMR spectra, the substance of hR_F 51 was identical with jacobine (*I*). The mother liquors after crystallization of jacobine hydrochloride were converted into free bases which on crystallization gave 340 mg of a substance of hR_F 57, m.p. 223–225°C (ethyl acetate). On the basis of the ¹H-NMR spectrum, it is a mixture of senecionine (*II*) and seneciophylline (*III*). The mixture was chromatographed on a column of cellulose powder (\varnothing 1.2 · 20 cm) in butanol saturated with 5% acetic acid according to ref.⁴, fractions amounting to 1 ml. The pure alkaloid of hR_F 58 was obtained from the fractions 33–37 (33 mg), the pure alkaloid of hR_F 56 from the fractions 44–49 (56 mg). The alkaloid of hR_F 58 had m.p. 242 to 244°C (ethyl acetate), $[\alpha]_D^{24} -53^\circ \pm 2^\circ$ (c 0.47 in chloroform). Senecionine (*II*) was reported⁶ to have m.p. 245°C, $[\alpha]_D -56^\circ$ (chloroform). The alkaloid of hR_F 56 had m.p. 216–217°C (ethyl acetate), $[\alpha]_D^{24} -140^\circ \pm 2^\circ$ (c 0.62 in chloroform). Seneciophylline (*III*) was reported⁶ to have m.p. 217°C, $[\alpha]_D -139^\circ$ (chloroform). The identity of the two isolated alkaloids with the authentic samples was confirmed by mixed melting point and ¹H-NMR spectra.

Isolation of Alkaloids from the Portion B

The plant was freely dried at 20°C (32 kg) and worked up in the same manner as sub *A*). The yield gave 17.1 g of acid and neutral substances, 36.4 g of a crude mixture of alkaloids (0.114% per dry weight), and 3.8 g of a crude mixture of alkaloids from N-oxides (0.012% per dry weight). According to thin-layer chromatography, the crude mixture of alkaloids contained alkaloids of hR_F 57, 51 and 26 in the ratio of c . 1 : 1 : 1. The crude mixture of alkaloids from N-oxides showed the same qualitative composition, the substances of hR_F 57 and 51 were, however, present in the ratio of c . 1 : 1, and the substance of hR_F 26 only in traces. The crude mixture of alkaloids (36.4 g) was extracted with 10% citric acid, the extract was washed with ether, made alkaline with ammonia and extracted with chloroform. The yield gave 28.9 g of a purified mixture of alkaloids (0.090% per dry weight) which crystallized from ethyl acetate to afford a crystalline mixture of the same composition as before crystallization. The mixture (3.5 g) was separated on a column (\varnothing 28 mm) of 100 g of Al₂O₃ (activity II — Reanal), fractions amounting to 10 ml. The fractions 31–522 (benzene up to chloroform–ethanol, 95 : 5) gave mixtures of alkaloids of hR_F 57 and 51 in various quantities. The pure alkaloid of hR_F 26 (1.02 g) was obtained from the fractions 532–546 (chloroform–ethanol, 90 : 10). Crystallization from ethanol yielded 609 mg of a substance of hR_F 26, m.p. 213–215°C, $[\alpha]_D^{24} -64^\circ \pm 2^\circ$ (c 0.29 in chloroform). Retrorsine (*V*) was reported⁶ to have m.p. 216°C, $[\alpha]_D -62^\circ$ (chloroform). The identity of the isolated substance with authentic retrorsine (*V*) was confirmed by the determination of the mixed melting point and a comparison of the IR and ¹H-NMR spectra.

The crystalline mixtures of the fractions 31–522 were combined and converted to hydrochlorides by treatment with ethanolic hydrogen chloride. Crystallization from ethanol gave 1.23 g of a substance which after conversion to a free base was found to be identical with jacobine (*I*). The mother liquors after crystallization of jacobine hydrochloride were converted to free bases, crystallization from ethyl acetate yielded 0.7 g of a substance of m.p. 228–230°C, identified on the basis of the ¹H-NMR spectra as a mixture of senecionine (*II*) and seneciophylline (*III*).

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