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## Structure and toxicity of the alkaloids of Russian comfrey (*Symphytum* × *uplandicum* Nyman), a medicinal herb and item of human diet

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*Summary.* Eight pyrrolizidine alkaloids of hepatotoxic type have been indentified in leaves of *Symphytum* × uplandicum. The combined alkaloids exhibit chronic hepatotoxicity in rats.

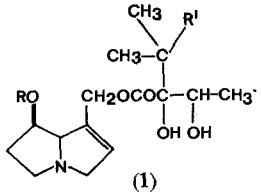
Russian comfrey (*Symphytum* + *uplandicum* Nyman), a hybrid of *S. officinale* L. and *S. asperum* Lepechin, is a leafy perennial grown as a garden herb and as a green feed for animals. In recent years it has been increasingly recommended by herbalists and organic gardeners as a medicinal herb, salad plant and 'green drink[2,3]. It is believed that several hundred thousand people now consume it in various forms and for many, comfrey has become a regular item of diet. As it is as member of the family Boraginaceae, which is noted for its many species containing hepatotoxic pyrrolizidine alkaloids[4a], Russian comfrey presents a possible dietary hazard. We report here the results of a chemical study of its alkaloids and a preliminary investigation of their chronic toxicity in rats.

Young leaves of a clone of Russian comfrey regarded as Bocking No. 4[2] were immersed in methanol immediately after collection and chopped into small pieces after soaking for 1 day. The extraction was completed in the usual way and the alkaloid fraction recovered after reduction of N-oxide with zinc dust[5].

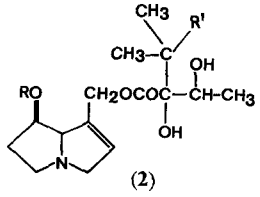
Countercurrent separation of the alkaloids resulted in a number of fractions which were characterized by nuclear magnetic resonance and gas chromatography-mass spectrometry (gc-ms). Distinction between possible diastereoisomers was achieved by these techniques together with hydrolysis and paper electrophoresis. Eight alkaloids were detected and identified as in table 1. Echimidine, symphytine, lycopsamine and intermedine are known alkaloids but 7-acetyllycopsamine, 7-acetylintermedine, symlandine, (7-angelyl-9-viridiflorylretronecine) and uplandicine (7-acetyl-9-echimidinylretronecine) have been characterized for the 1st time and will be described in detail elsewhere. The total alkaloid content of different leaf samples varied in the range 0.01–0.15%. Pedersen[6] has reported 0.9% in a Danish variety and obtained mass spectral evidence that the main constituents had the gross structures represented by lycopsamine, 7-acetyllycopsamine and echimidine. The traditional medicinal comfrey, *S. officinale*, has been shown to contain echimidine and symphytine[7,8] and 2 other alkaloids with the gross structures of lycopsamine and 7-acetyllycopsamine[6].

Echimidine has an acute $LD_{50}$ of 200 mg/kg by i.p. injection in rats[4b] and repeated injections at the rate of 20 mg/kg, thrice weekly, produce moderate chronic liver damage within 18 weeks[4c]. The $LD_{50}$ of symphytine for mice is approximately 300 mg/kg by i.p. injection[7]. Single oral dosage of rats with a mixture of lycopsamine and intermedine from *Amsinckia intermedia* caused acute death at a dose rate of 1500 mg/kg, acute CNS effects at 1000 mg/kg and chronic liver damage, typical of pyrrolizidine alkaloid poisoning, at 500 mg/kg[9]. Pancreatic islet cell

Table 1. Alkaloids in leaf of *Symphytum* × *uplandicum*



(1)



(2)

| | | Percent of total alkaloid |
|---|---|---|
| Lycopsamine | (1), R = H, R′ = H | 13 |
| 7-Acetyl-lycopsamine | (1), R = $CH_3CO$, R′ = H | 32 |
| Symphytine | (1), R = $CH_3-C(CH_3)=CH-CO$, R′ = H | 5 |
| Symlandine | (1), R = $CH_3-CH=C(CH_3)-CO$, R′ = H | 5 |
| Intermedine | (2), R = H, R′ = H | 3 |
| 7-Acetylintermedine | (2), R = $CH_3CO$, R′ = H | 6 |
| Echimidine | (1) or (2), R = $CH_3-CH=C(CH_3)-CO$, R′–OH | 24 |
| Uplandicine | (1) or (2), R–$CH_3CO$, R′ = OH | 12 |

Table 2. Effects of comfrey alkaloids on rat liver

| Alkaloid | Dose (mg/kg) | No. of rats | Mitotic index | Nuclear population density | Total plasma proteins (mg/ml) | Albumin/ globulin | Plasma enzymes m units/ml GOT | GLDH |
|---|---|---|---|---|---|---|---|---|
| Comfrey alkaloids | 71 | 5 | 0 | 47±1.1 | 26.9±3.9 | 0.78±0.13 | 141±18 | 9.5 (2 rats) |
| Lasiocarpine | 10.3 | 6 | 0.02±0.02 | 57.2±2.0 | 38.5±3.3 | 1.09±0.12 | 125±4 | |
| Platyphylline | 33.7 | 5 | 1.52±0.02 | 113.4±3.4 | 54.2±0.9 | 1.47±0.05 | 45±5 | |
| Saline controls | - | 6 | 1.70±0.47 | 91±3.7 | 55.1±0.9 | 1.45±0.06 | 41±2 | 1.9±1.1 |

Total comfrey alkaloids, lasiocarpine, platyphylline or saline were given 3 times weekly i.p. to groups of hooded rats, both male and female, commencing when the rats were 14 days old. After 3 weeks' treatment blood was withdrawn by cardiac puncture, the heparinized plasma assayed for total plasma proteins, albumin/globulin ratios, GOT and GLDH by standard methods[20,21] and portions of liver prepared for histological examination. Nuclear population density represents the mean number of parenchymal nuclei per microscopic field (magnification × 630) and the mitotic index the number of mitoses per 1000 parenchymal cell nuclei. Values given are means ± SE. The values for comfrey alkaloids and lasiocarpine differ from those of the saline controls (Kruskal-Wallis test) with significance levels of $p < 0.01$ for mitotic index, albumin/globulin ratios and GOT levels and $p < 0.001$ for nuclear population density and total plasma proteins.

tumours occurred in 3 of 15 rats that survived these doses for over 2 years[10]. Being a diester of similar gross structure to echimidine and symphytine, symlandine would be expected to approximate these alkaloids in $LD_{50}$. On the other hand, a comparison of heliotrine and 7-acetylheliotrine[11] suggests that the new bases with a 7-acetoxy grouping will show toxicities close to those of the corresponding alkaloids with a 7-OH group.

These considerations suggest that the acute $LD_{50}$ of the total alkaloid fraction of Russian comfrey should be about 550 mg/kg for rats. However, in the single-dose screening test[12], in which doubling doses are administered by i.p. injection to 2-week-old rats, peracute (non-hepatic) deaths occurred with a dose of 284 mg/kg. A dose of 142 mg/kg caused temporary distress shown by jerky, inco-ordinated movements but no deaths, while lower doses had no apparent effect. No histological liver damage was evident in rats killed 4 weeks after injection. An inability to demonstrate liver lesions within this time was found previously with alkaloids of low hepatotoxicity[11] and was ascribed to the intervention of peracute toxicity preventing the administration of an adequate dose level.

Rats given repeated doses of the total alkaloid of Russian comfrey at the rate of 71 mg/kg i.p., thrice weekly, died within 3–4 weeks with severely impaired liver function, but only mild histological damage consisting mainly of necrosis of scattered single parenchymal cells or groups of a few cells. There was an increase in mean nuclear diameter of parenchymal cells due mainly to a shift towards a higher proportion of nuclei in the upper normal size range; this effect was reflected in the nuclear population density (table 2), which was reduced to about half that of the controls. Mitotic activity in parenchymal cells was greatly reduced (table 2). Although these qualitative changes are insufficiently developed for a definite diagnosis, the values differ significantly from the controls and are consistent with early pyrrolizidine alkaloid damage.

In contrast to this mild histological damage, impairment of some aspects of liver function was severe (table 2). Both the total plasma protein and the albumin/globulin ratio were reduced by 40% or more. On the other hand, the increase in the levels of liver enzymes (aminoaspartate transaminase and glutamic dehydrogenase) was relatively small, a finding consistent with the necrosis of only isolated parenchymal cells as shown histologically. In most rats these changes were accompanied by the development of ascites and/or ventral s.c. oedema. As shown in table 2, similar biochemical and histological effects were produced by a comparable course of injections of the strongly hepatotoxic alkaloid, lasiocarpine, at a dose level which was estimated to be approximately the same proportion of an acute $LD_{50}$. The effects were not produced by the non-hepatotoxic alkaloid, platyphylline, given at a dose level equal to half that causing peracute death[11]. Reduced liver function was also detected after thrice weekly administration of Russian comfrey alkaloids at levels of 17.8 and 8.9 mg/kg for 3 weeks.

These results suggest that under certain conditions of administration of pyrrolizidine alkaloids impairment of liver function may be sufficient to cause death before the changes that characterize the histology of the chronic disease become apparent; the morphological changes induced directly by the alkaloids and the reparative responses to tissue damage either have insufficient time to develop or may possibly be suppressed by the continued presence of the alkaloid. These deaths are clearly distinguishable from the acute deaths with massive hepatic necrosis that occur within about 1 week of the administration of high doses of alkaloid. They are more closely related to the chronic disease and are indicative of selective interference with parenchymal cell function that is sustained for periods of some weeks without loss of cell viability.

In confirmation of their hepatotoxicity, continued administration of the alkaloids of Russian comfrey at a dose level of 17.8 mg/kg, thrice weekly for 9 weeks, was found to cause frank megalocytosis of the hepatocytes.

The susceptibility of humans to pyrrolizidine alkaloid poisoning is now well established[13–15] although only 1 estimate is available of a low-level intake leading to toxicity. In an outbreak involving wheat contaminated with seed of *Heliotropium popovii* subsp. *gillianum*, the intake of alkaloid which caused severe liver disease in 2 years was estimated at about 2 mg/person/day[14] or approximately 30–40 µg/kg/day. The rate of ingestion of Russian comfrey alkaloids may exceed this level since several leaves may be eaten per day and the level of alkaloid in the material available to us was approximately 1 mg/leaf. Dose rates of similar magnitude have produced chronic lesions in experimental animals, although with alkaloids of higher acute toxicity. Thus a monocrotaline intake of about 80 µg/kg/day, occasioned by feeding *Crotalaria retusa* seed at the level of 0.004% in the diet for 20 weeks, produced megalocytosis of the liver and kidney in pigs[16]. The hepatotoxic pyrrolizidine alkaloids which have been adequately tested are also carcinogenic in experimental animals[17]. The lowest effective dose rates have not been established, lasiocarpine having produced tumours in rats at dose rates equivalent to 350 and 170 µg/kg/day[18,19]. Root and leaf of *Symphytum officinale* have recently been shown to be carcinogenic in rats when fed at rates down to 0.5% and 8% of diet, respectively[22].

The pyrrolizidine alkaloid content of Russian comfrey provides grounds for concern at the human consumption of this plant, especially by children because of the greater

sensitivity of young animals to the effects of this type of alkaloid[23,24]. The lack of reports of toxicity of this plant despite claims of dietary use over many years[2] is not necessarily an indicator of safety. The effects of such alkaloids are cumulative and overt damage may be long delayed, thus preventing association with the plant cause.

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## Activity of precocene analogs on *Locusta migratoria migratorioides* (R. and F.)[1]

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*11 July 1979*

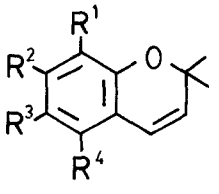
*Summary.* Several analogs of precocene have been synthesized and evaluated as potential inhibitors of juvenile hormone on *Locusta migratoria.* Only 5,7-dimethoxy-2,2-dimethylchromene was active; its $ED_{50}$ and $LD_{50}$ were measured and compared to those of precocene I and II.

Although considerable interest has been devoted to insect juvenile hormones, molting hormones (ecdysones) and synthetic juvenile hormone mimics, relatively little work has been done on inhibitors of hormones regulating metamorphosis in insects. This subject has been reviewed recently by Slama[2].

Bowers et al.[3] found that chromene derivatives extracted from the plant *Ageratum houstonianum* have anti-juvenile hormone like effects. Demethoxy-ageratochromene (7-methoxy-2,2-dimethylchromene) **1** and ageratochromene (6,7-dimethoxy-2,2-dimethylchromene) **2** possess anti-allatotropic properties and induce precocious metamorphosis in several insect orders. Because of their properties, they have been renamed precocene I and precocene II. Activity of precocenes has been already reported on 2 members of Orthoptera: *Locusta migratoria*[4] and *Schistocerca gregaria*[5]. We wish to report here the synthesis and evaluation of precocene analogs as potential inhibitors of juvenile hormone on *Locusta migratoria.*

*Methods and materials.* The substituted 2,2-dimethylchromenes (**3–8**) have been prepared following the general method of Hlubucek[6]: Substituted phenols (from Aldrich Co.) reacted with 3-chloro-3-methylbut-1-yne in refluxing acetone in the presence of anhydrous potassium carbonate and potassium iodide to produce in high yields the corresponding aryl $\alpha,\alpha$-dimethylpropargyl ethers. Thermal rearrangement of the ethers in boiling diethylaniline proceeded smoothly to give the chromenes in good yields. The 3-chloro-3-methylbut-1-yne was readily obtained from commercially available 3-hydroxy-3-methylbut-1-yne[7].

Crowded locusts were kept in a regime of 12 h light and 12 h darkness at 28 °C. Relative humidity was maintained at 45%. The compound to be tested was dissolved at different concentrations in spectral grade acetone so that each insect received 10 µl of the solution. This solution was applied topically on the ventral part of abdomen on 4th instar nymphs 1–24-h-old. Effects were recorded every day. Tests were performed on groups of 20 locust nymphs and repeated 2 times.




| | $R^1$ | $R^2$ | $R^3$ | $R^4$ |
|---|---|---|---|---|
| **1** | H | OMe | H | H |
| **2** | H | OMe | OMe | H |
| **3** | H | H | H | H |
| **4** | H | H | OMe | H |
| **5** | OMe | H | H | H |
| **6** | H | OMe | H | OMe |
| **7** | H | $O-CH_2-$ | O | H |
| **8** | H | OMe | OMe | OMe |