VALEPOTRIATES IN TISSUE CULTURES OF NINE DIFFERENT VALERIANACEAE SPECIES IN COMPARISON TO LITERATURE DATA OF THE INTACT PLANTS

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Abstract.—Tissue cultures of nine different species of Valerianaceae plants (*Valeriana officinalis* L., *V. wallichii* DC., *V. alliariifolia* Vahl, *V. sambucifolia* Mik, *Fedia cornucopiae* (L.) Gaertn., *Centranthus ruber* (L.) DC., *Valerianella dentata* (L.) Poll., *V. ella coronata* (L.) DC., and *V. ella locusta* (L.) Later) have been analyzed for their valepotriates. The amount of valepotriates was compared to literature data of intact plants. In some cases (e.g. *Fedia*) the relative amount in tissue cultures was higher than in the intact plant.

Several plants within the family of the Valerianaceae show a sedative effect. According to pharmacological investigations (1), at least part of this effect is due to a group of iridoid substances referred to by Thies as valepotriates (2); cf. fig. 1.

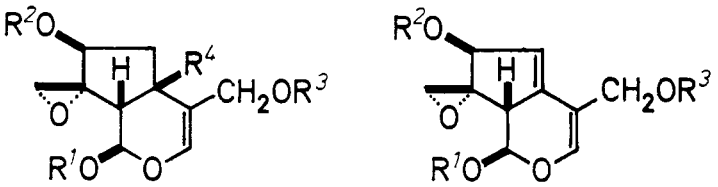

Fig. 1. Structure of the main valepotriates.

Didrovaltrate: R^1, R^3=IV; R^2=Ac; R^4=H.
Isovaleroxyhydroxydidrovaltrate: R^1=IIV; R^2=IV; R^3=Ac; R^4=OH.
Valtrate: R^1, R^2=IV; R^3=Ac.
Acevaltrate: R^1=AIV; R^2=IV; R^3=Ac.
Isovaltrate: R^1, R^3=IV; R^2=Ac.

Ac =-OOC-CH_3 (acetate).
IV =-OOC-CH_2-$CH(CH_3)_2$ (isovaleric acid).
AIV=OOC-CH_2-$C(CH_3)_2$ O·CO·CH_3 (β-acetoxyioxysovaleric acid).
IIV =-OOC-CH-$CH(CH_3)_2$ O·CO-CH_2-$CH(CH_3)_2$ (α-isovaleroyloxyisovaleric acid).

In a previous paper, we reported that root organ cultures and undifferentiated tissue cultures of *V. wallichii* contained the main valepotriates, valtrate, acevaltrate, and didrovaltrate, known from the intact plant (3). These results are not only of theoretical interest but could also be of practical use (4) because of the increasing need for crude drug and isolated valepotriates. In order to see whether valepotriates are also accumulated in vitro by other Valerianaceae plants, we initiated tissue cultures of different species, from which we could get either fresh parts of the whole plant or viable seeds. Up to now, nine different species have been grown as agar cultures, two of which were also established as cell suspension cultures.

EXPERIMENTAL

Initiation and maintenance of tissue cultures.—*V. wallichii* and *V. officinalis* were initiated from stem cuttings, all other cultures from aseptically grown seeds, according to the classical methods (5). Cultivation began on a basal Murashige and Skoog's medium (6) with different amounts of α-naphthaleneacetic acid (1–5 mg/liter) and kinetin (1–2 mg/liter). In

all cases coconut-water (10%) was added for the initiation and first passages. It was omitted, however, in the following subcultures. The cultures were maintained in 12 h/d light at room temperature (22±2° C). They were subcultured every 3 to 4 weeks. Early growth responses of the explants are described elsewhere in detail (7).

EXTRACTION.—The cell mass of 10 flasks was harvested and freeze-dried three weeks after subculturing. One g of freeze-dried tissue was extracted three times with 50 ml of CH_2Cl_2 in a homogenizer. The extracts were filtered and evaporated to dryness. The residue was dissolved in 2 ml of ethylacetate and used for tlc and quantitative evaluation.

THIN-LAYER CHROMATOGRAPHY.—*a) for quantitative estimation:* layer, Silica Gel 60_{F254}; solvent, *n*-hexane-ethylmethylketone (80:20, v/v); detection, (1) uv 254; (2) dinitrophenylhydrazine (8).

b) for qualitative estimation: solvent, dichloromethane-ethyl acetate-acetone (48:1:1, v/v/v) (9); other conditions as above. Reference substances: valtrate, didrovaltrate, acevaltrate, and isovaleroxydidrovaltrate.[1]

QUANTITATIVE EVALUATION OF THE VALEPOTRIATES.—The quantitative evaluation of valtrate and acevaltrate was done according to Stahl and Schild (10). Both valepotriates were separated by tlc and measured by direct photometry. We were able to evaluate didrovaltrate in a similar way. A calibration curve for each substance was established from the plate used for the test. The values represent the average of three assays. Variation coefficient; valtrate: 2.4%; acevaltrate: 2.8%; didrovaltrate 4%.

SPECTROPHOTOMETER.—PM QII (Zeiss); remission (Pr-M); valtrate and acevaltrate at 256 nm; slit, 1 x 6 mm; amplification, II/A/1/9; didrovaltrate at 208 nm; slit, 2 x 6 mm; amplification, II/F/10/5.

RESULTS AND DISCUSSION

All but one tissue culture produced valepotriates in detectable amounts. Only the cells of *V. officinalis* did not contain valepotriates. This may be due to the fact that valepotriates are only found in the roots of this plant, while the other plants contain the substance in the foliage as well as in the roots (11). The biogenetic capability of green tissue cultures resembles that of the foliage of the intact plant rather than its roots.

The capability of the eight other cultures to produce valepotriates is not only expressed during the first passages, but persists for a longer period. Eight species have now been cultivated for at least one and a half years. Tissue cultures of *V. wallichii* have been cultivated for three and a half years, and they still produce valepotriates.

The different cultures vary in their spectrum as well as in the quantitative amount of valepotriates. The qualitative comparison of the valepotriates with the solvent system of Van Meer and Labadie (9) showed that valtrate can be detected in fair amounts in all cultures. Isovaltrate is only present in *V. wallichii* in fairly good amounts. In *Fedia, V. ella dentata, V. ella coronata,* and *V. ella locusta* only traces are found. The occurrence of isovaltrate in *Centranthus ruber* is uncertain. The highest amounts of didrovaltrate are found in *Fedia* and, to a lesser degree, in *V. wallichii.* Acevaltrate is found in all *Valerianella* species and in *V. alliariifolia.* Isovaleroxydidrovaltrate is shown to be present in *Fedia, V. ella dentata,* and *V. ella locusta.*

Many tissue cultures of medicinal plants produce secondary compounds known from the original plant. However, the amount of these compounds in culture is in most cases lower than in the plant (4, 12, 13). In order to classify the amount of valepotriates of our cultures, we compared them with literature data of the roots of intact plants (see table 1). As most of these data were obtained by direct photometry of tlc-plates (10), we chose the same method. Though valtrate and

[1]The authors want to thank Dr. Thies, Kali Chemie, Hannover, Germany, for authentic samples of the valepotriates.

isovaltrate can be detected by tlc (9), the resolution does not allow a separate estimation by direct photometry. As seen in table 1, the amount of valepotriates in *V. wallichii*, *V. ella dentata*, and *V. ella coronata* lies within the range of the roots of intact plants. For *Fedia* and *V. ella locusta*, we even found higher amounts than in the intact plants. Besides *V. officinalis*, which did not contain any valepotriates, only *V. alliariifolia* had much less valepotriates than the drug.

TABLE 1. Relative amount of the main valepotriates in tissue culture of different Valerianaceae species. Values are expressed in % dry weight (a); these are compared to literature data for the roots of the corresponding intact plants (b).

Tissue culture species	Valtrate/Isovaltrate		Didrovaltrate		Acevaltrate	
	a	b	a	b	a	b
Valeriana officinalis	—	0.05–1.0[a]	—	0.01	—	0.01
Valeriana wallichii	0.66	0.6[b]	0.10	1.4	0.34	0.4
Valeriana alliariifolia	0.36	2.5[a]	0.06	0.3	0.23	—
Valeriana sambucifolia	0.40	—	traces	—	0.02	—
Fedia cornucopiae	1.38	0.6[a]	0.30	0.01	0.08	0.01–0.02
Centranthus ruber	0.63	—	0.06	—	0.10	—
Valerianella dentata	0.80	0.2–0.6[a]	traces	traces	0.19	0.05
Valerianella coronata	0.61	0.5–0.7[a]	traces	traces	0.45	0.05
Valerianella locusta	0.88	0.05[a]	0.05	traces	0.21	traces

[a]See ref. (10).
[b]See ref. (14).

These results show that tissue cultures of *Valeriana* species could be a useful source for valepotriates. For commercial use, however, the amount of these products must still be increased. In order to find the best conditions for biomass and secondary product formation, we will try to isolate variant strains of one species.

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