

## SAPONINS IN *CYCLAMEN* SPECIES: CONFIGURATION OF CYCLAMIRETIN C AND STRUCTURE OF ISOCYCLAMIN\*

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**Key Word Index**—*Cyclamen*; Primulaceae; saponin; sapogenin; chemotaxonomy.

**Abstract**—Five sapogenins and three saponins were identified in bulbs of 14 cytologically defined *Cyclamen* species and their structures were determined by means of mass,  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopy. In addition to the known products of hydrolysis, primulagenin A was found, and cyclamiretin C was shown to be identical with cyclamigenin C. The new saponin isocyclamin was identified as  $3\beta$ -{ $O$ - $\beta$ -D-glucopyranosyl-(1-6)-[ $O$ - $\beta$ -D-xylopyranosyl-(1-2)]- $O$ - $\beta$ -D-glucopyranosyl-(1-4)-[ $O$ - $\beta$ -D-glucopyranosyl-(1-2)]- $\alpha$ -L-arabinopyranosyl}-16 $\alpha$ -hydroxy-13 $\beta$ , 28-epoxy-olean-30-al.

### INTRODUCTION

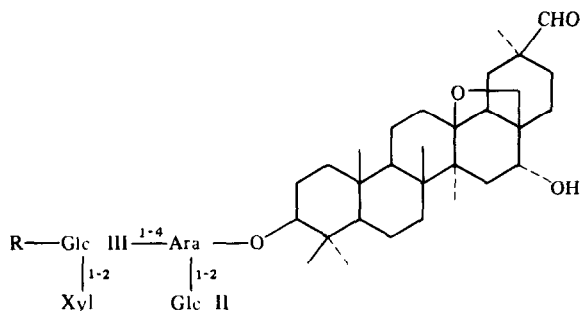
The genus *Cyclamen* contains 19 species [1]. Investigations are reported only on saponins and sapogenins in bulbs of *Cyclamen europaeum* (= *C. purpurascens*) and to a smaller extent on *C. neapolitanum* and *C. graecum*. The saponin mixture of these species consists mainly of cyclamin, desglucocyclamin I (Table 1) and small amounts of desglucocyclamin II [2–4]. Acid hydrolysis of the crystallized saponin mixture revealed cyclamiretin A and the artifacts cyclamiretin B, C and D [5–8], whereas the remaining saponin portion gave the cyclamigenins A<sub>1</sub>, A<sub>2</sub>, B, C, D and E [9–11].

### RESULTS AND DISCUSSION

TLC comparison of 14 species (see Experimental) showed three main saponins: the known compounds desglucocyclamin I (1) and cyclamin (2) and a new substance (3) with higher polarity than cyclamin. Desglucocyclamin II was detected in small amounts only. The main saponins desglucocyclamin I, cyclamin and the new saponin were then isolated from bulbs of *Cyclamen graecum* by repeated CC and the pure substances were characterized by means of FABMS,  $^{13}\text{C}$  NMR and  $^1\text{H}$  NMR spectroscopy. The new saponin (3) was identified by comparing the  $^{13}\text{C}$  NMR spectra of these three compounds (Table 1) as  $3\beta$ -{ $O$ - $\beta$ -D-glucopyranosyl-(1-6)-[ $O$ - $\beta$ -D-xylopyranosyl-(1-2)]- $O$ - $\beta$ -D-glucopyranosyl-(1-4)-[ $O$ - $\beta$ -D-glucopyranosyl-(1-2)]- $\alpha$ -L-arabinopyranosyl}-16 $\alpha$ -hydroxy-13 $\beta$ , 28-epoxy-olean-30-al, and was therefore called isocyclamin.

Acid hydrolysis of the saponin complex from bulbs of *C. coum* subsp. *coum* and *C. africanum* gave after repeated CC cyclamiretins A (4), C (5), D (6) and cyclamigenin A<sub>1</sub> (7) as main products. Cyclamiretin C with yet unknown configuration of C-30 was also prepared by acid treat-

ment of pure cyclamiretin A.  $^1\text{H}$  NMR spectra proved that the C-30 proton was  $\beta$ -positioned and thus cyclamiretin C must be identical in structure with cyclamigenin C [8, 11]. Therefore the name cyclamigenin C should not be used any longer. A further sapogenin in much smaller quantity than cyclamiretin A was identified as primulagenin A (8), not previously found in *Cyclamen*. Considering the results of earlier investigations about saponins of Primulaceae [12], it is likely that this compound is present in the genuine glycosides as protoprimulagenin A. TLC comparison of the sapogenins from the various *Cyclamen* species showed qualitatively and quantitatively identical spectra. There were, however, differences in the quantities of the main saponins: in *C. balearicum*, *C. repandum*, *C. rhodeum*, *C. creticum*, *C. persicum* and *C. graecum* an approximately equal amount of cyclamin, desglucocyclamin I and isocyclamin was found; in *C. pseudibericum*, *C. neapolitanum* and *C. purpurascens* cyclamin dominated, and in *C. mirabile*, *C. cilicicum*, *C. coum* subsp. *coum*, *C. cyprium* and *C. africanum*, desglucocyclamin I was the main compound. The given relations were shown to be independent of the stage of development of the *Cyclamen* plants investigated.



- |   |                    |                 |
|---|--------------------|-----------------|
| 1 | Desglucocyclamin I | R = H           |
| 2 | Cyclamin           | R = Glc 1 (1→3) |
| 3 | Isocyclamin        | R = Glc 1 (1→6) |

\*Part of the thesis of G. Reznicek, University of Vienna, in preparation.

Table 1.  $^{13}\text{C}$  NMR data of desglucocyclamin I (1), cyclamin (2) and isocyclamin (3)

Sapogenin moiety Cyclamiretin A			Sugar moiety				
			1	2	3		
C	$\text{CD}_3\text{OD}$	Pyridine- $d_5$	$\text{CD}_3\text{OD}$	$\text{CD}_3\text{OD}$	$\text{CD}_3\text{OD}$	Pyridine- $d_5$	
1	40.5	39.5	ara	1 105.5	105.5	105.5	105.6
2	27.4	26.7		2 80.2	80.2	79.8	79.8
3	91.5	89.0		3 74.4	74.2	74.2	73.7
4	40.8	39.8		4 78.0 <sup>a</sup>	78.1 <sup>a</sup>	78.0 <sup>a</sup>	78.8 <sup>a</sup>
5	57.0	55.7		5 67.5	67.2	67.3	67.5
6	18.9	17.9	xyl	1 107.3	105.7	107.2	107.7
7	33.9	33.5		2 76.2	76.1	76.0	76.4
8	43.6	42.5		3 78.0 <sup>a</sup>	78.0 <sup>a</sup>	78.0 <sup>a</sup>	78.4 <sup>a</sup>
9	51.5	50.4		4 71.3	70.0	71.3	71.1
10	37.3	36.9 <sup>a</sup>		5 65.9	65.5	65.7	65.0
11	20.0	19.1					
12	35.3	34.4	glc I	1 —	104.5	104.4	104.4
13	88.3	86.3		2 —	75.3	75.2	75.1
14	44.9	44.0		3 —	78.1 <sup>a</sup>	77.1	76.9
15	38.0	36.9 <sup>a</sup>		4 —	71.6	71.6	71.5
16	79.8	79.8		5 —	78.0 <sup>a</sup>	77.1	77.0
17	45.5	44.6		6 —	62.5	62.8	62.6
18	54.1	53.3					
19	31.2	30.5	glc II	1 104.6	104.5	104.5	104.8
20	49.2	48.3		2 75.9	75.6	75.8	76.1
21	34.1 <sup>b</sup>	32.7 <sup>b</sup>		3 77.7 <sup>a</sup>	77.8 <sup>a</sup>	77.8 <sup>a</sup>	78.3 <sup>a</sup>
22	33.0 <sup>b</sup>	32.4 <sup>b</sup>		4 71.1	71.1	71.0	70.7
23	28.7	28.1		5 77.7 <sup>a</sup>	77.8 <sup>a</sup>	77.8 <sup>a</sup>	78.2 <sup>a</sup>
24	17.0 <sup>c</sup>	16.3 <sup>c</sup>		6 63.5	63.3	63.3	63.0
25	16.9 <sup>c</sup>	16.6 <sup>c</sup>	glc III	1 104.9	104.9	105.1	104.8
26	19.1	18.5		2 85.1	82.0	85.0	85.3
27	20.4	19.7		3 78.0 <sup>a</sup>	87.4	77.6 <sup>a</sup>	77.9 <sup>a</sup>
28	78.6	78.7		4 72.2	71.9	72.0	71.8
29	24.5	24.1		5 78.1 <sup>a</sup>	78.1 <sup>a</sup>	78.0 <sup>a</sup>	77.7 <sup>a</sup>
30	209.4	207.5		6 62.8	65.5	70.3	70.1

ara:  $\alpha$ -L-arabinopyranosyl, xyl:  $\beta$ -D-xylopyranosyl, glc:  $\beta$ -D-glucopyranosyl.<sup>a-c</sup> Data vertically exchangeable.

## EXPERIMENTAL

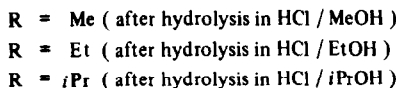
**Plant material.** The *Cyclamen* plants were cultivated in a greenhouse, morphological and cytological identification was performed according to ref. [1]. Voucher specimen are deposited in the Institute of Botany, University of Vienna. Plant origins: *C. africanum* Boiss. et Reut. (2n=68, unknown origin); *C. balaricum* Willk. (2n=20, 2 origins Mallorca/Spain); *C. cilicicum* Boiss. et Held. (2n=30, Alanya/Turkey, Beyşehir/Turkey); *C. coum* Mill. subsp. *coum* (2n=30, unknown origin); *C. creticum* Hildebr. (2n=22, Crete/Greece); *C. cyprium* Schott and Kotschy (2n=30, Cyprus); *C. graecum* Link (2n=84, Crete/Greece, Lesbos/Greece, Navplion/Greece); *C. mirabile* Hildebr. (2n=30, Izmir/Turkey); *C. neapolitanum* Tenore (2n=34, Nazilli/Turkey, Orgrazden/Bulgaria, Sterea/Greece); *C. persicum* Mill. (2n=48, Karatas/Turkey, Cyprus, Cesme/Turkey); *C. pseudibericum* Hildebr. (2n=30, Karatepe/Turkey, Dül dül dagı/Turkey); *C. purpurascens* Mill. = *C. europaeum* (2n=34, unknown origin); *C. repandum* Sibth. et Smith. (2n=20, Parnon oros/Greece, Mistras/Greece); *C. repandum* var. *rhodense* Meikle (2n=20, Rhodos/Greece).

**TLC comparison of saponins and sapogenins.** For comparison of saponins 30  $\mu\text{l}$  of liquid were taken directly from bulbs (three bulbs of all species were investigated three times during one

period of growth) by means of a glass capillary and used directly for TLC on silica gel 60 F<sub>254</sub> MERCK, 0.25 mm; mobile phase:  $\text{CHCl}_3$ -MeOH-H<sub>2</sub>O (63:39:10); detection by spraying with EtOH-conc. H<sub>2</sub>SO<sub>4</sub>-anisaldehyde (17:2:1) and heating the plates to 150° for 15 min.; *R<sub>f</sub>*-values (purple spots): desglucocyclamin II (0.53), desglucocyclamin I (0.49), cyclamin (0.41), isocyclamin (0.36).

The sapogenins were prepared by hydrolysis of 30  $\mu\text{l}$  liquid of bulbs (10 ml 50% EtOH and 0.165 ml conc. HCl under C<sub>6</sub>H<sub>6</sub>, 3 hr, boiling H<sub>2</sub>O bath). The C<sub>6</sub>H<sub>6</sub> solution was concd under red. pres. and used for TLC on silica gel 60 F<sub>254</sub> Merck, 0.25 mm; mobile phase:  $\text{CHCl}_3$ -Me<sub>2</sub>CO (9:1); detection as above. *R<sub>f</sub>*-values: cyclamiretin A (0.5); cyclamigenin A<sub>1</sub> (0.45); cyclamiretin C = cyclamigenin C (0.33); cyclamiretin D (0.18)—all purple; primulagenin A (0.25) red-purple.

**Isolation of saponins.** 123 g crushed bulbs of *C. graecum* were extracted  $\times 3$  with 200 ml boiling H<sub>2</sub>O, the aq. layer was extracted several times with *n*-BuOH. From the residue of the organic layer (3.1 g) 11 mg desglucocyclamin I and 12 mg isocyclamin were isolated by repeated CC over silica gel Merck (0.063–0.200 mm) with mobile phases  $\text{CHCl}_3$ -MeOH-H<sub>2</sub>O (13:8:2) and (13:6:2). 22 mg cyclamin were obtained by repeated CC over silica gel 60 (as above) and over silica gel TSC WOELM with H<sub>2</sub>O satd *n*-BuOH as mobile phases. FABMS: cyclamin



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# REFERENCES

1. Saunders, D. E. (1975) *Cyclamen, the Genus in the Wild and in Cultivation*, Alpine Garden Society's Bulletin, revision March 1975.
2. Tschesche, R., Mercker, H. J. and Wulff, G. (1969) *Liebigs Ann. Chem.* **721**, 194.
3. Anil, H. (1977) *Bitki* **4**, 179.
4. Anil, H. (1977) *Doga* **1**, 148.
5. Barton, D. H. R., Hameed, A. and McGhie, J. F. (1962) *J. Chem. Soc. (London)* 5176.
6. Tschesche, R., Inchaurrondo, F. and Wulff, G. (1964) *Liebigs Ann. Chem.* **680**, 107.
7. Tschesche, R., Striegler, H. and Fehlhaber, H. W. (1966) *Liebigs Ann. Chem.* **691**, 165.
8. Harvala, C. and Hylands, P. J. (1978) *Planta Med.* **33**, 180.
9. Dorchai, R. O. and Thomson, J. B. (1965) *Tetrahedron Letters* **26**, 2223.
10. Dorchai, R. O. and Thomson, J. B. (1968) *Tetrahedron* **24**, 1377.
11. Dorchai, R. O., Rubalcava, H. E. and Thomson, J. B. (1968) *Tetrahedron* **24**, 5649.
12. Kitagawa, I., Matsuda, A. and Yosioka, I. (1968) *Tetrahedron Letters* **51**, 5377.
13. Budzikiewicz, H., Wilson, J. M. and Djerassi, C. (1963) *J. Am. Chem. Soc.* **85**, 3688.
14. Glombitza, K. W. and Kurth, H. (1987) *Planta Med.* **53**, 548.