

barrier for glucose, but probably formed a complete permeability barrier for dithane. Had there not been a permeability barrier for dithane, the dithane-resistant mutant would have been expected to be defective in PS II activity, leading to the development of an auxotrophy for a fixed carbon source, as in the previously reported methylamine-resistant mutant of *N. muscorum*<sup>17</sup>. Dithane-resistance was thus thought to be a case of permeation mutation. Further experiments to confirm the presence of a permeability barrier for dithane in the dithane-resistant mutant of *N. muscorum* are in progress. Experiments with glucose suggested the existence of an active glucose-transport system in *N. muscorum* as well as its dithane-resistant mutant strain.

The present findings give a clear indication that it would be possible to isolate mutant strains of blue-green algae resistant to other pesticides as well, which can grow, multiply and continue fixing nitrogen in fields treated with those pesticides.

- 1 Thanks are due to Council of Scientific and Industrial Research, Govt. of India, New Delhi-110001, for providing financial assistance to A.V. in the form of a Post-Doctoral Research Fellowship.
- 2 Department of Botany, L.N.M. University, Darbhanga-846004, India.

- 3 T.F. Armstrong, F.M. William and P. Donald, *Weed Sci.* 21, 354 (1973).
- 4 A.D. Dodge, *Sci. Prog., Oxford* 62, 447 (1975).
- 5 G.E. Fogg, W.D.P. Stewart, P. Fay and A.E. Walsby, in: *The Blue-green Algae*, p.279. Ed. G.E. Fogg. Academic Press, London 1973.
- 6 W.D.P. Stewart, *A. Rev. Microbiol.* 27, 283 (1973).
- 7 R.N. Singh, in: *Role of Blue-green Algae in Nitrogen Economy of Indian Agriculture*, p.75. Indian Council of Agricultural Research, New Delhi 1961.
- 8 A. Vaishampayan, H.R. Singh and H.N. Singh, *Biochem. Physiol. Pfl.* 173, 410 (1978).
- 9 S.R.A. Fisher and F. Yates, *Statistical Tables*. Oliver and Boyd Publ., 1957.
- 10 H.N. Singh and A. Vaishampayan, *Envir. exp. Bot.* 18, 87 (1978).
- 11 K.H. Buchel, *Pestic. Sci.* 3, 89 (1972).
- 12 N.I. Bishop, *Biochim. biophys. Acta* 27, 205 (1958).
- 13 N.M. Weare and J.R. Benemann, *Arch. Mikrobiol.* 93, 101 (1973).
- 14 R.Y. Stanier, in: *The Biology of Blue-green Algae*, p.501. Ed. N.G. Carr and B.A. Whitton. University of California Press, Berkeley and Los Angeles 1973.
- 15 R.A. Pelroy, R. Rippka and R.Y. Stanier, *Arch. Mikrobiol.* 97, 69 (1974).
- 16 D.S. Hoare and R.B. Moore, *Biochim. biophys. Acta* 109, 622 (1965).
- 17 A. Vaishampayan and H.N. Singh, *Biochem. Physiol. Pfl.* 176, 621 (1981).

### Chemical subdivisions within the genus *Arctostaphylos* based on flavanoid profiles<sup>1</sup>

K. E. Denford

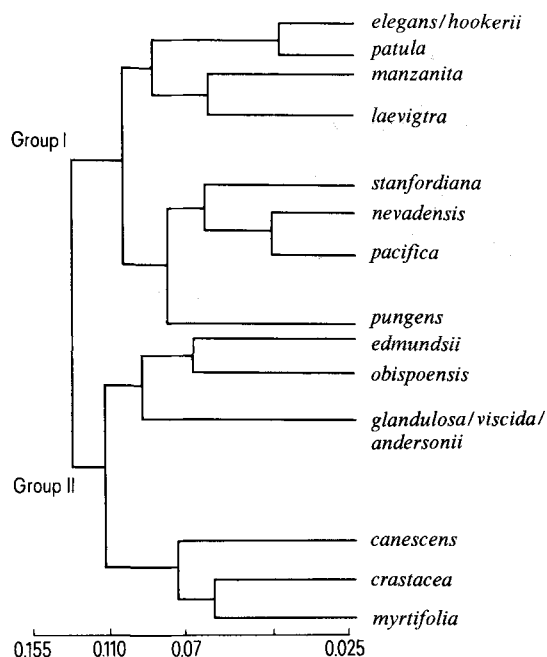
Botany Department, University of Alberta, Edmonton (Alberta, Canada T6G 2E9), 9 April 1981

**Summary.** The flavonoid glycosides from 227 populations representing 20 species of *Arctostaphylos* have been identified. Certain glycosides are of values in subdividing the genus into discrete chemically related groups. A single linkage computer analysis shows the existence of subdivisions based both on oxidation levels of the flavonoids as well as glycoside variation. The ability to form 7-O-glycosides appears to be restricted and could be of future value in the identification of hybrids between those taxa capable of 7-O-glycoside synthesis and those unable to do so.

*Arctostaphylos* is a mainly North West American genus of woody plant sometimes divided into about 100 taxa<sup>2</sup>. Many of these taxa are described and delimited by a few trivial, if not questionable characters and subsequently the genus appears to be taxonomically difficult to evaluate. In all probability there are about 25–50 distinguishable taxa with definite delimitable characters.

Previous studies on *Arctostaphylos uva-ursi* (L.) Spreng. have indicated the usefulness of flavonoids as phyto-geographic and taxonomic markers within the genus<sup>3,4</sup>. Furthermore, studies have been carried out on the distribution of flavonoid aglycones within the genus. Of 41 species, subspecies and varieties evaluated; 10/41 produced myricetin and quercetin derivatives; 21/41 produced myricetin, quercetin and kaempferol derivatives, and the remainder produced quercetin and kaempferol derivatives alone<sup>5</sup>. Several workers have suggested that levels of B-ring hydroxylation in flavonoids is indicative of primitive versus advanced phylogenetic standing<sup>6</sup>. It would appear therefore that within the genus *Arctostaphylos* there are both 'primitive' and 'advanced' taxa – those producing trihydroxy and dihydroxy derivatives but no monohydroxy flavonoids (i.e., kaempferol) and those not producing trihydroxy derivatives (i.e., myricetin).

As part of a continuing study of this genus a total of 227 populations representing the taxa in the table were evaluated for their flavonol glycoside profiles as examples of biochemically 'primitive' vs 'advanced' forms. Voucher specimens were deposited in the University of Alberta Vascular Plant Herbarium.



Phenogram of 20 taxa of *Arctostaphylos* on the basis of a single linkage analysis of their flavonoid-glycoside pattern.

Distributions of flavonoid glycosides in certain members of the genus *Arctostaphylos*

	M. 3-O-rha	M. 3-O-ara	M. 3-O-glc	M. 3-O-rhaglc	Q. 3-O-gal	Q. 3-O-glc	Q. 3-O-rha	Q. 3-O-ara	Q. 3-O-diara	Q. 3-O-rhaglc	Q. 3-O-diglc	Q. 7-O-glc	K. 3-O-rha	K. 3-O-gal	K. 3-O-ara	K. 3-O-rhaglc	K. 3-O-diglc	Number of populations
<i>A. elegans</i> Jepson	+		+	+	+	+		+	+	+	+							7
<i>A. hookerii</i> Don	+		+	+	+	+		+	+	+	+							6
<i>A. patula</i> H. B. K.	+		+	+	+	+	+	+	+	+	+							6
<i>A. manzanita</i> Parry	+	+	+	+	+	+	+	+	+	+	+							21
<i>A. manzanita</i> ssp. <i>laevigata</i> (Eastw.) Munz.	+		+	+	+	+	+	+	+	+	+							6
<i>A. stanfordiana</i> Parry	+			+	+	+		+	+	+	+							6
<i>A. pacifica</i> Roof	+			+	+	+		+	+	+	+	+						14
<i>A. nevadensis</i> Gray	+			+	+	+	+	+	+	+		+						6
<i>A. pungens</i> H. B. K.	+			+	+	+		+		+								19
<i>A. pungens</i> H. B. K. var. <i>montana</i> (Eastw.) Munz.	+			+	+	+		+		+								7
<i>A. obispoensis</i> Eastw.					+	+				+	+		+		+	+		6
<i>A. edmundsii</i> Howell					+	+		+		+	+		+		+	+		8
<i>A. glandulosa</i> Eastw.					+	+				+			+		+	+		23
<i>A. glandulosa</i> Eastw. var. <i>crassifolia</i> Jeps.					+	+				+			+		+	+		14
<i>A. viscida</i>					+	+				+			+		+	+		7
<i>A. andersonii</i> Gray					+	+				+			+		+	+		20
<i>A. andersonii</i> Gray <i>pallida</i> (Eastw.) Adams ex McMinn.					+	+				+			+		+	+		21
<i>A. canescens</i> Eastw. var. <i>candidissima</i> (Eastw.) Munz.					+	+				+	+		+		+	+		17
<i>A. crataegae</i> Eastw. var. <i>rosei</i> (Eastw.) McMinn.					+	+				+	+	+	+	+	+	+		7
<i>A. myrtifolia</i> Parry					+	+				+	+	+	+	+	+	+		6

Air dried pressed leaves of each taxon were exhaustively extracted with 80% aq. ethanol. Excessive chlorophyll was removed by partitioning with multiple aliquots of petrol-ether and after concentration, paper chromatography and Sephadex column fractionation of the subsequent extracts showed a total of 17 flavonoids distributed within the 20 taxa (a minimum of 6 populations and a maximum 23 populations per taxon). Standard methods were used to establish flavonoid identities<sup>6-8</sup> (UV, rfs., fluorescence, spectrophotometry and co-chromatography with known standards).

4 myricetin, 8 quercetin and 5 kaempferol glycosides were identified (table). Of the quercetin glycosides identified were 3 ubiquitous (quercetin 3-O-galactoside, 3-O-glucoside and 3-O-rhamnoglucoside) (table). Within the myricetin-producing taxa myricetin 3-O-rhamnoside, and 3-O-rhamnoglucoside were common to all taxa as were their counterparts in the kaempferol-producing group (kaempferol 3-O-rhamnoside and 3-O-rhamnoglucoside). Quercetin 3-O-arabinoside was found in all myricetin-producing taxa, but in only 1 kaempferol-producing taxon *A. edmundsii* (table).

Also of note is the fact that although quercetin 3-O-galactoside is ubiquitous, its counterpart myricetin 3-O-galactoside was not detected in any of the taxa investigated, and kaempferol 3-O-galactoside was found in only 1 taxon (*A. crataegae* var. *rosei*). Furthermore, although there is a discontinuity in distribution of flavonoid glycosides, certain taxa, although morphologically distinguishable from each other, have identical profiles, e.g., *A. elegans* - *A. hookerii*, also *A. viscida* - *A. andersonii* and *A. pallida*, all the 3 latter taxa being biochemically identical (table).

The distribution of 7-O-glycosides is restricted to only 4 of the taxa investigated (*A. stanfordiana*, *A. nevadensis*, *A. cras-*

*tacea* and *A. myrtifolia*) and consequently could be of value as a taxonomic marker in assessing the origins of any hybrids within the genus. Of possible importance in the systematics of the genus is the presence of certain glycosides that appear to be key characters in the formation of sub-divisions within the *Arctostaphylos* complex. Quercetin 3-O-diglucoside (probably the sophoroside) is a major dividing character in the subdivision of the 'manzanita' members of group I whereas kaempferol 3-O-arabinoside is a key character in separating the 'glandulosa' taxa from the remaining members of group II.

A 'single linkage' analysis of the distribution of flavonoid glycosides isolated allows a separation of the taxa investigated into 2 major groups based on hydroxylation levels as previously discussed<sup>10</sup>. No taxa so far investigated appears to 'bridge the gap' between these 2 groups. Further studies are at present continuing to determine if this holds true.

- 1 Supported by the National Sciences and Engineering Research Council of Canada.
- 2 P. V. Wells, *Madrono* 19, 193 (1968).
- 3 K. E. Denford, *Experientia* 29, 939 (1973).
- 4 J. G. Packer and K. E. Denford, *Can. J. Bot.* 52, 743 (1974).
- 5 K. E. Denford, unpublished.
- 6 J. B. Harborne, *Biochemistry of Phenolic Compounds*. Academic Press, London and New York 1964.
- 7 T. J. Mabry, D. R. Markham and M. B. Thomas, *The Systematic Identification of Flavonoids*. Springer-Verlag, New York 1970.
- 8 J. B. Harborne, *Comparative Biochemistry of the Flavonoids*. Academic Press, London and New York 1967.
- 9 P. H. A. Sneath and R. R. Sokal, *Numerical Taxonomy*. Freeman Cooper, San Francisco 1973.
- 10 J. B. Harborne, *Phytochemical Phylogeny*. Academic Press, London and New York 1970.