Flavonoids of *Arctostaphylos uva-ursi* (Ericaceae)

The genus *Arctostaphylos* (Ericaceae) contains 25 species, the majority of which are restricted in distribution to Western North America. *Arctostaphylos uva-ursi* (L.) Spreng, (bearberry) is widely distributed in North America, especially Canada, and has been shown to be of two cytologically and morphologically distinguishable varieties (var. *adenotricha* Fern. and Macbr. $2n = 26$ and var. *coactilis* Fern. and Macbr. $2n = 52$).[1,2] *A. uva-ursi* var. *adenotricha* would appear to be restricted in its distribution to North America, whereas var. *coactilis* is also found in Europe and Asia[1-3].

Recent studies have demonstrated that a third variety of *A. uva-ursi* is restricted in its distribution to elevated regions of the Canadian Rockies and Alaska[2]. This variety has the same chromosome number as var. *coactilis* but is morphologically distinct, and is referred to in this paper as 'stipitate'.

As part of a continuing study of this species complex, 50 samples of both vars. *adenotricha* and *coactilis*, as well as 6 samples of the rarer 'stipitate' form were assayed for flavonoids.

Fresh leaves of each variety of *Arctostaphylos uva-ursi* were extracted with 80% ethanol. Excessive chlorophyll was removed with multiple aliquots of petrol-ether. Two-dimensional paper chromatography of hydrolyzed and non-hydrolyzed extracts showed a total of eleven flavonoids. The major aglycone present was quercetin and the minor aglycone myricetin. Standard methods were used to establish flavonoid identities[4,5] (UV, Rf's, fluorescence, spectrophotometry and direct comparison with known standards).

Five quercetin monoglycosides were identified after purification and hydrolysis (Table) and 2 myricetin monoglycosides as well as 2 quercetin diglycosides. It is of interest to note that all the flavonoids encountered are flavonols showing a somewhat primitive biochemical profile[6]. The absence of certain flavonoids in the stipitate form (myricetin 3–0 arabinoside, quercetin 3–0 arabinoside and quercetin 7–0 glucoside) may well indicate a phylogenetic difference between the latter and its more commonly distributed relatives.

The restricted phytogeographic distribution of the 'stipitate' form especially its occurrence in suspected glacial refugia of the Rocky Mountains and Alaska[7], has led the author to suspect that biochemical markers, such as flavonoids, might be of value in establishing plant refugial boundaries. Such studies are at present under way.

Résumé. Dans trois variétés d'*Arctostaphylos uva-ursi*, j'ai trouvé les flavanoides suivants: arabinoside-3, glucoside-3, galactoside-3, diglucoside-3, rutinoside-3, rhamnoside-3, glucoside-7-quercetine and arabinoside-3, glucoside-3-myricetine. J'ai déduit une corrélation entre la distribution limitée de certains d'entre ces flavonoides et les localités, en Amérique du Nord qui ont échappé a la glaciation.

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Distribution of flavonoids in *Arctostaphylos uva-ursi*

	Varieties		
	adenotricha	coactilis	stipitate
Myricetin	+	+	+
M 3-0 arabinoside	+	+	—
M 3-0 glucoside	+	+	+
Quercetin	+	+	+
Q 3-0 galactoside	+	+	+
Q 3-0 glucoside	+	+	+
Q 3-0 rhamnoside	+	+	+
Q 3-0 arabinoside	+	+	—
Q 3-0 diglucoside	+	+	+
Q 3-0 rhamnoglucoside	+	+	+
Q 7-0 glucoside	+	+	—

1 E. Hultén, *Flora of Alaska and neighbouring territories* (Stanford University Press, California 1968).
2 J. G. Packer, Can. J. Bot. *45*, 1767 (1967).
3 M. L. Fernald and J. F. MacBride, Rhodora *16*, 211 (1914).
4 T. J. Mabry, K. R. Markham and M. B. Thomas, *The Systematic Identification of Flavonoids* (Springer-Verlag, New York 1970).
5 J. B. Harborne, *Biochemistry of Phenolic Compounds* (Academic Press, London and New York 1964).
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8 Acknowledgment. This investigation was supported in part by the NRC of Canada, Grant No. A6663.

The Role of Glycine in the Biosynthesis of Steroids[1]

We have previously reported[1] that [2-^{14}C] glycine, [3-^{14}C] serine, [S-methyl-^{14}C] methionine, [^{14}C] formate and other progenitors of 'one-carbon units' can act as efficient substrates for the fungus *Cochiobolus miyabeanus* to produce radioactive ophiobolin B – a sesterterpene which can be derived from mevalonic acid. Interestingly, [1-^{14}C] glycine led to poor incorporation of radioactivity indicating that deamination of glycine to acetic acid did not occur to any appreciable extent. We believe that [3-^{14}C] pyruvic acid or equivalent is an intermediate in the biosynthetic pathway[1].

We report in the Table our observations on the biosynthesis of isoprenoids by *Saccharomyces cerevisiae*[2] in presence of labeled amino acids. Since the side chain of ergosterol carries an 'extra' methyl group known to be derived from one carbon donors such as methionine, it is essential to discount the radioactivity carried by this methyl group in evaluating the incorporation of radioactivity. Ergosterol from yeast purified by preparative TLC was subjected to slow oxidation with concentrated nitric acid; an aromatic acid-1-methyl-2, 3, 5, 6-tetracarboxy-

1 a) Studies on Biosynthesis. Part VI. For Part V, see A. K. Bose, K. S. Khanchandani and B. L. Hungund, Experientia, *27*, 1403 (1971). b) Presented at the 164th National Meeting of the American Chemical Society, New York, August, 1972.
2 H. P. Klein, N. R. Eaton and J. C. Murphy, Biochim. biophys. Acta *13*, 591 (1954).

Isoprenoid biosynthesis by *Saccharomyces cerevisiae*[2]

Substrate added	Spec. act. of amino acid, (dpm/mM: I_s)	Spec. act. of metabolite, (dpm/mM: I_m)	Incorporation of ^{14}C (%)	Overall isotope dilution[8] I_s/I_m
[2-^{14}C] glycine (30 μC, 10 mg)	499×10^6	ergosterol (as digitonide): 260×10^3	0.05	2000
		squalene (as hexahydrochloride): 200×10^3	0.04	2500
		saponifiable fraction: 955 cpm/mg		
DL-[3-^{14}C] serine (40 μC) + L-serine (10 mg)	936×10^6	ergosterol (as digitonide): 260×10^3	0.02	4500
		squalene (as hexahydrochloride): 250×10^3	0.02	3700
[1-^{14}C] glycine (40 μC, 10 mg)	667×10^6	no appreciable counts in ergosterol or squalene; saponifiable fraction: 590 cpm/mg		

benzene- was obtained which is known[3] to be derived from the carbons of the A, B, and C rings of ergosterol. In one biosynthetic experiment[4] using [2-^{14}C] glycine (spec. act., 166×10^6 dpm/mM), ergosterol (spec. act., 129×10^4 dpm/mM, sp. incorporation 3.5%) was degraded to this acid (spec. act. 268×10^2 dpm/mM). In view of the known efficiency of the methylene carbon of glycine for methyl transfer in biosynthetic reactions, it is not surprising that most of the radioactivity of ergosterol was accounted for by the 'extra methyl group'. What is significant is that the acid possessed appreciable radioactivity demonstrating thereby that C-2 of glycine was being used efficiently enough in isoprenoid synthesis in spite of competition with side-chain methylation. [1-^{14}C] glycine conferred little reactivity on ergosterol although the fatty acid fraction was labeled nearly as efficiently by this substrate as by [2-^{14}C] glycine.

In subsequent experiments with yeast an even more direct link between glycine and steroid biosynthesis was found. Squalene, the established precursor of many steroids, could be isolated from the metabolites produced by *S. cerevisiae*. Purified through the crystalline hexahydrochloride, this metabolite showed substantial radioactivity (spec. act., 460×10^3 dpm/mM) in an experiment with [2-^{14}C] glycine. In contrast, when [1-^{14}C] glycine was used very little activity was present in the ergosterol or the squalene produced; the saponifiable fraction, however, was appreciably radioactive (see Table).

In another series of experiments rat liver homogenate preparations[5] were incubated with various ^{14}C-labeled substrates; cholesterol from these experiments was found to be radioactive. In case of glycine, the methylene carbon was incorporated nearly 10 times more efficiently than the carboxy carbon; [3-^{14}C] serine also was found to label cholesterol efficiently[6].

In view of our observations reported here on fungi, yeast and rat liver preparations, it is evident that biosynthesis of steroids and terpenoids from amino acids that can produce 'one carbon units' is a general phenomenon. It is therefore important to study the role of amino acids from the protein part of diet in the formation of cholesterol independent of the contribution from fats and carbohydrates. Currently cholesterol is considered to be strongly implicated in atherosclerosis and heart diseases. The recent report by CASPI et al.[7] that in vivo incorporation of the S-methyl carbon of methionine into cholesterol takes place in normal and tumorous rats is further evidence for the hitherto unrecognized pathways from amino acids to isoprenoids.

Zusammenfassung. Während der Biosynthese von Cholesterol mit homogenisierter Rattenleber wird [2-^{14}C] des Glycins viel besser eingebaut als [1-^{14}C]. *Saccharomyces cerevisiae* produziert radioaktives Squalen (ausser Ergosterol mit Radioaktivität des Ringsystems) mit [2-^{14}C] Glycin und mit [3-^{14}C] Serin, aber nicht mit [1-^{14}C] Glycin.

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[8] The 'overall isotope dilution' does not take into account the number (n) of sites in the metabolite that can bear the label; the true 'isotope dilution' will be n times larger.
[9] The support of this research by Stevens Institute of Technology and Sandoz Foundation is gratefully acknowledged. We wish to thank Drs. P. T. FUNKE, M. S. MANHAS, P. K. BHATTACHARYYA, M. ANCHEL and H. LEVEY for valuable discussions and help with some of the experiments.

Occurrence of N-Imidazolepropionylhistamine in the Soft Tissues of the Philippine Gastropod *Drupa concatenata* Lam.

The only N-acylated histamine derivative so far found in the living organism is N-acetylhistamine which has been traced in the urine of several mammals, and in some tissues, including nervous tissue[1-5].

This communication describes the occurrence of large amounts of N-imidazolepropionylhistamine in methanol extracts of the total soft tissues of *Drupa concatenata* Lam., a gastropod of the Philippines.