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SHORT COMMUNICATION

STUDIES ON THE METHYLATION OF CYMAROSE IN *ADONIS VERNALIS*

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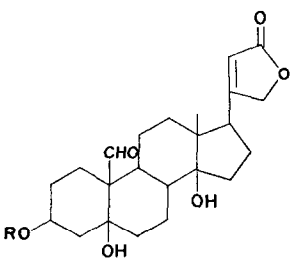
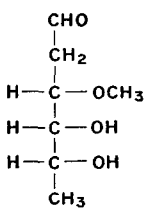
Abstract—It could be shown by *in vivo* feeding experiments that *S*-adenosyl methionine (SAM) was an active methyl group donor for the formation of the 3-*O*-methyl group of D-cymarose in the cardiac glycoside cymarin. Glucose on the other hand was transformed into the main carbon skeleton of D-cymarose and it was only used to a small extent for the formation of the 3-*O*-methyl group.

INTRODUCTION

THE CARDIAC glycoside cymarin (I), which occurs in *Adonis vernalis* and different *Strophanthus* species, is known to contain the 2,6-dideoxy-3-*O*-methyl sugar, D-cymarose (II).[1,2] The biological methylation of sugars and polysaccharides has already received some attention. The methyl groups of the *C*-methyl sugar mycarose [3] and of 4-*O*-methyl glucuronic acid in hemicelluloses[4] were shown to be transferred from *S*-adenosyl methionine (SAM) after the complete *C*-skeleton of the sugars had been formed. The methyl group of 6-*O*-methylglucose of *Mycobacterium tuberculosis* was also shown to be transferred from SAM but only at the triose level.[5] It was therefore of interest to find out whether the 3-*O*-methyl group of the 2,6-dideoxysugars found in cardiac glycosides originates from the same precursor.



I Cymarin
R=II



II Cymarose

RESULTS AND DISCUSSION

S-adenosyl methionine (methyl-^{14}C), D-glucose-1-^{14}C and D-glucose-U-^{14}C were fed to *Adonis vernalis* plants and allowed to metabolize for different time intervals after which

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[3] H. PAPE, R. SCHMID and H. GRIESEBACH, *Europ. J. Biochem.* **10,** 429 (1969).
[4] H. KAUSS, *Phytochem.* **8,** 985 (1969).
[5] F. A. LORNITZO and D. S. GOLDMAN, *Biochem. Biophys. Res. Commun.* **35,** 215 (1969).

TABLE 1. INCORPORATION OF SAM (methyl-^{14}C), GLUCOSE-U-^{14}C AND GLUCOSE-1-^{14}C INTO THE GLYCOSIDE CYMARIN OF *Adonis vernalis* PLANTS

Substrate fed to the plant	Time of incubation (h)	Dose fed to the plant (μM/kg dry wt.) (×10³)	μm of substrate incorporated into 1 mg cymarin (×10⁶)	μm of substrate incorporated into cymarose* (×10⁶)	μm of substrate incorporated into aglycon* (×10⁶)	μm of substrate incorporated into 3-*O*-methyl group* (×10⁶)	μm of substrate incorporated into C-atoms 1-6 of cymarose† (×10⁶)
SAM (methyl-^{14}C)	2	28	5·2	4·3	0·9	4·0	0·1
	8	34	29	19	6 0	16	1·5
	24	21	67	46	21	34	4·0
Glucose-U-^{14}C	2	3·5	19	12	4·0	—	11
	8	3·9	37	29	6·0	1·0	23
	24	3·75	56	36	18	3·0	32
Glucose-1-^{14}C	2	16	57	46	11	—	46
	8	18	98	73	14	0·1	66
	24	19·5	110	81	19	2·0	72

* Per mg cymarin isolated.
† After cleavage of the 3-*O*-methyl group.

the glycosides were extracted and separated. The cymarose-containing glycoside cymarin was hydrolysed and the distribution of radioactive label determined (Table 1). Radioactivity was measured in total glycoside, aglycon, cymarose, 3-*O*-methyl group and after splitting of the 3-*O*-methyl group, in the remaining sugar skeleton.

S-adenosyl methionine was shown to be a more effective precursor than glucose for the 3-*O*-methyl group in cymarose. When glucose-U-^{14}C was fed to the plant, radioactive label was only found in the main sugar chain, when short times of incubation were used. But with SAM a transfer of label from the methyl-^{14}C group into the 3-*O*-methyl group of the sugar cymarose could be observed even after 2 hr: at this time the main carbon skeleton of the sugar remained almost inactive. Only after an incubation of 24 hr was a considerable incorporation of radioactivity from glucose-U-^{14}C into the 3-*O*-methyl group of cymarose observed, indicating that glucose was rearranged and partially transformed via intermediates to the 3-*O*-methyl group. But the label of the *C*-atom in the methyl group is very small compared to that found in the residual 6 *C*-atoms of the main sugar chain. After 24 hr incubation with SAM, the carbon atoms one to six in cymarose carried a small amount of label as well. But radioactivity in the *C*-atom of the 3-*O*-methyl group was considerably higher than in all six *C*-atoms of the skeleton together.

The experiment with glucose exclusively labelled in *C*-1-position was to show whether this *C*-atom was transferred to the 3-*O*-methyl group after rearrangement of the main carbon chain. This does not seem to be the case since even after a 24-hr incubation the amount of labelling in the 3-*O*-methyl group in cymarose is small compared to the experiments with glucose labelled in all *C*-atoms.

The question is still open whether the attachment of the 3-*O*-methyl group occurs after the glycosidic linkage is formed or before the sugar is attached to the aglycon.

EXPERIMENTAL

Radioactive material was obtained from Radiochemical Centre Amersham: *S*-adenosyl-methionine (methyl ^{14}C, 58 mc/mM) (SAM), D-glucose-1-^{14}C (54 mc/mM) and D-glucose-U-^{14}C (320 mc/mM). Radioactivity was measured in a Tri-Carb liquid scintillation spectrometer and with the aid of a Berthold (LB 2722) gas flow TLC-scanner.

Outdoor grown *Adonis vernalis* plants were harvested during and shortly after flowering. Labelled substrates were administered to the fresh cut plants following the method previously described.[6] The isolation of the glycosides was carried out by the methods of Stoll and Kreis[7] and of Fuchs *et al.*[8] The second method gave a higher yield of glycosides. The glycoside complex was separated by silica gel TLC (Merck aluminum Sheets) with the solvent system EtOAc–pyridine–H_2O, (4:1:5, v/v) upper phase. Radioactive cymarin was eluted from the plates with $CHCl_3$–MeOH (1:1). The hydrolysis of the glycoside was performed as described by Rees.[9] The oxidative cleavage of the 3-*O*-methyl group of cymarose was carried out following the method of Harrison.[10]

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