Gas–liquid chromatography of plant glycosides

Methods for separating and identifying microquantities of plant glycosides are of great importance for work in phytochemistry, pharmacognosy and chemotaxonomy. Thin-layer chromatography, which is most extensively used, does not possess the high sensitivity that is often desirable.

The successful separation of naturally occurring compounds such as aglycones by means of gas–liquid chromatography has been reported by several authors[1–7], but does not appear to have been achieved with plant glycosides. Recently SWEELEY and his coworkers[6] were able to separate the two plant glycosides aesculin and phlorizin on a gas chromatograph.

Gas chromatographic separation of seventeen plant glycosides, which are treated with hexamethyldisilazane and trimethylchlorosilane, has now been carried out on column containing SE-30 silicone rubber (0.75 %) on Chromosorb W (80–100 mesh). This paper describes the results obtained with a representative variety of simple phenolic, coumarin, isocoumarin, isoflavone, anthraquinone, cyanogenetic, isothiocyanate and monoterpene glycosides.

Experimental

Materials. Many of the plant glycosides used were available in this laboratory, but the author is very much indebted to Prof. H. INOUE for the monotropein, catalposide and arbutin derivatives, to Dr. TAKIDO for emodinglucoside, and to Mr. M. MATSUO for sinigrin. Hexamethyldisilazane was obtained from Peninsular Chemresearch, U.S.A. Trimethylchlorosilane was obtained from Applied Science Laboratories, Inc. Solvents were reagent grade.

The column packing 0.75 % of SE-30 silicone rubber on 80–100 mesh Chromosorb W was obtained from Applied Science Laboratories, Inc., U.S.A.

Procedure. The standard conditions for trimethylsilylation used were as follows: (a) 10 mg of plant glycoside was treated with 1 ml of anhydrous pyridine, 0.2 ml of hexamethyldisilazane and 0.1 ml of trimethylchlorosilane. (b) When sufficient material was not available, 1 mg of plant glycoside was treated with 0.1 ml of anhydrous pyridine, 0.1 ml of hexamethyldisilazane, 0.05 ml of trimethylchlorosilane, or (c) 1 mg of plant glycoside was dissolved in 0.2 ml of anhydrous tetrahydrofuran and treated with 0.4 ml of hexamethyldisilazane and 0.1 ml of trimethylchlorosilane.

The reaction was carried out in a glass-stoppered vial. The mixture resulting from (a) and (b) was shaken vigorously for about 30 sec, and was then allowed to stand for 10 min. In the case of (c), the reaction mixture was left standing overnight at room temperature.

0.1 to 1 μl of the resulting mixtures were used for injection into the gas chromatograph.

Gas chromatography. A Shimazu Model GC-1B gas chromatograph equipped with a hydrogen flame ionization detector was used in this work. The column containing 0.75 % SE-30 silicone rubber on Chromosorb W (80–100 mesh) consisted of stainless steel U tubes, 2.25 m in length and having an inner diameter of 4 mm. The trimethylsilyl ethers of plant glycosides were introduced with a Hamilton microliter syringe.

Results and discussion

A number of plant glycosides gave single sharp peaks which appears to indicate the absence of decomposition or, if any, a little decomposition such as that with sinigrin. The gas chromatograms and retention times are shown in Table I and Fig. 1. No difference in retention times could be observed when either pyridine or tetrahydrofuran was used as the solvent for trimethylsilylation.

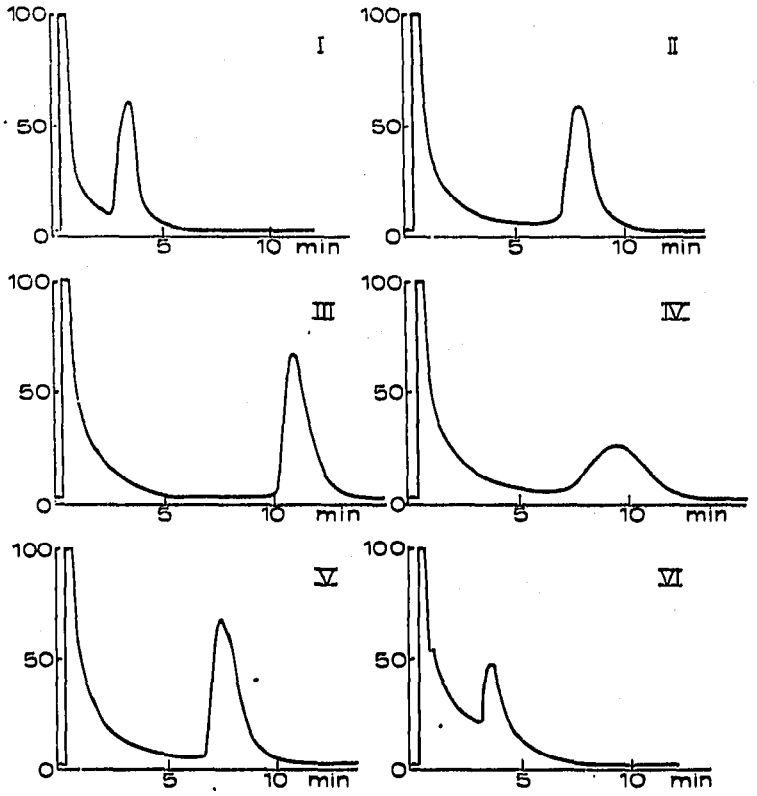

Fig. 1. Gas chromatograms of plant glycosides as the trimethylsilyl ethers. I = salicin (t_R = 3.7); II = fraxin (8.5); III = bergenin (11.0); IV = amygdalin (9.0); V = paconiflorin (7.4); VI = sinigrin (3.4). For the operating conditions see the footnote to Table I.

Five simple phenolic glycosides were gas chromatographed. Arbutin, glucoside of hydroquinone, occurs widely in Ericaceae (*Arctostaphylos**) and other families. Homoarbutin has been isolated from Pirolaceae (*Pirola*). Arbutin can be easily separated from homoarbutin and isohomoarbutin methyl ethers used as reference compounds. Salicin, phenolic glucoside of salicyl alcohol, was first found in Salicaceae (*Salix*). Gaultherin, glucoside of salicylic acid methyl ester, was found in Ericaceae (*Gaultheria*). Salicin and gaultherin both gave good peaks. Aesculin, 6-glucoside of aesculetin, has been reported in Hippocastanaceae (*Aesculus*), fraxin, 8-glucoside of fraxetin, has been found in Oleaceae (*Fraxinus*), and daphnin, 7-glucoside of daphnetin, was distributed in Thymeleaceae (*Daphne*). The three coumarin glycosides mentioned above can be more easily separated in this way than by thin-layer chromatography[8]. Bergenin is one of the isocoumarin derivatives and its

* The name in parentheses shows the representative genus, from which plant glycoside has been isolated.

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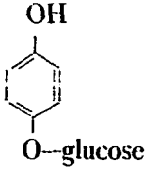
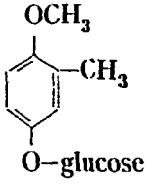
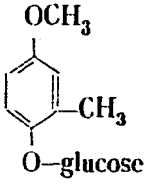
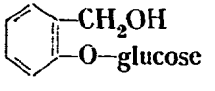
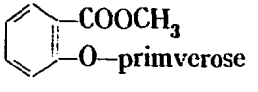
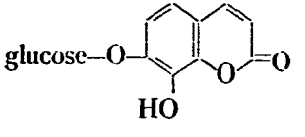
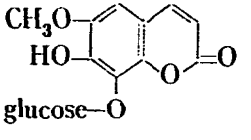
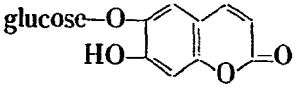
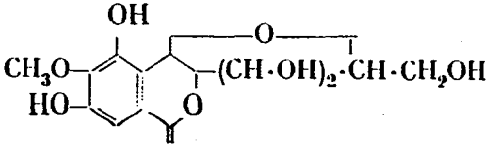
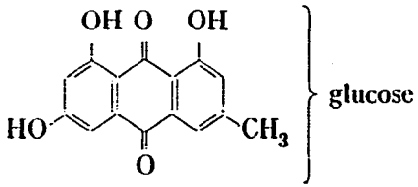
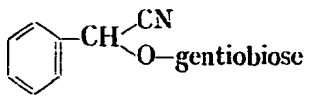
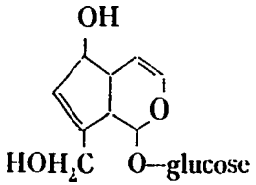
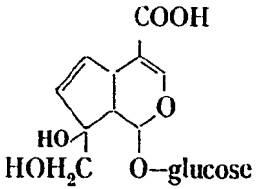
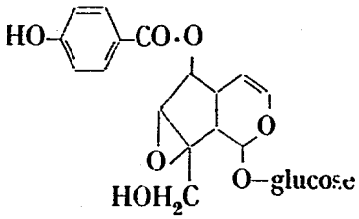
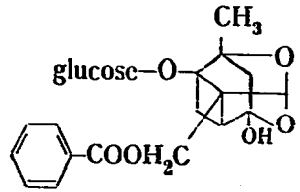
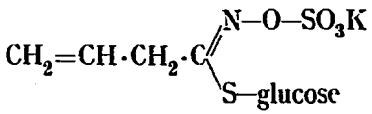
Group	Compound	Structure	Column temperature		
			188°	203°	243°
Simple phenols	Arbutin		8.2	4.2	1.2[a]
	Homoarbutin methyl ether		7.6	3.5	—
	Isohomoarbutin methyl ether		7.0	3.3	—
	Salicin		—	3.7	1.3[a]
	Gaultherin		—	5.8	—
Coumarin glycosides	Daphnin		—	7.4	3.0[a]
	Fraxin		—	8.5	3.3[a]
	Aesculin		—	10.5	3.9[a]
Isocoumarin glycosides	Bergenin		11.0	11.0	2.8[a]

		glucose–O–			
Anthraquinone glycosides	Emodin glucoside		—	—	8.0
Cyanogenetic glycosides	Amygdalin		—	—	9.0
Other glycosides	Aucubin		—	2.9	1.7
	Monotropein		—	7.6	2.6
	Catalposide		—	26.9[b]	11.1[m], 7.4[s]
	Paeoniflorin		—	24.9[b]	7.4
	Sinigrin		3.4[m], 1.8[s]	1.9[m], 1.1[s]	—

Conditions: stainless steel column 2.25 m long, 4 mm I.D.; packing 0.75% SE-30 on Chromosorb W (80–100 mesh); N_2 flow rate 122.4 ml/min at column temp. 188°, 119 ml/min at 203°, 102 ml/min at 243°; a = 110.5 ml/min at 243°. Detector temp. 265°; flash heater temp. 305°.

peaks indicated approximately 500 theoretical plates. Daidzin, 7-glucoside of daidzein and genistin glucoside isolated from Leguminosae have got well-defined peaks. Amygdalin, one of the cyanogenetic glycosides, appears to be most stable to heat. Monoterpene glycosides, such as aucubin from Cornaceae (*Aucuba*), monotropein from Pirolaceae (*Monotropa*), catalposide from Bignoniaceae (*Catalpa*) and paeoniflorin from Paeoniaceae (*Paeonia*) gave good peaks whose retention times increase in proportion as the number of C-atoms in the compounds increases as shown below: aucubin (C_{15}, $t_R = 1.7$), monotropein (C_{16}, $t_R = 2.6$) and catalposide (C_{22}, $t_R = 11.1$). The gas chromatogram of sinigrin showed a major peak with an additional minor peak. At a column temperature of 175° three peaks, namely $t_R = 6.35$ (main), 4.8 (minor), 3.1 (minor), were observed.

Because of its great sensitivity and resolving power, it is suggested that this method will be useful for the analysis of medicinal plants and their constituents.

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Faculty of Pharmaceutical Sciences,
University of Tokyo (Japan)

TSUTOMU FURUYA

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The importance of column material in the gas chromatography of isocyanides

In gas chromatography, interferences caused by the material of the tube itself have received little notice until recently. For the analysis of chlorinated pesticides, quartz[1] or glass[2] tubes have been recommended instead of metal; glass is preferred for phosphorus compounds[3]. Glass is also safer for steroid analysis although metal may be used in a properly designed system[4]. On the other hand, all-glass is the only way to handle some pyrrolizidine alkaloids[5]. In these cases it appears that the metal surface catalyses decomposition.

We have observed that with aliphatic isocyanides glass tubing is satisfactory