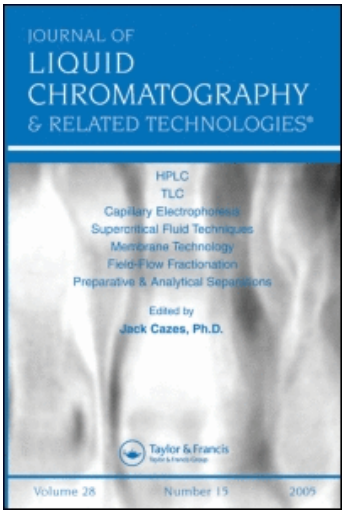

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Cheng-Ji Xu[a]; Jiann-Tsyn Lin[a]
[a] Plant Physiology and Chemistry Research Unit Western Regional Research Center Agricultural Research Service U.S. Department of Agriculture, Albany, California

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COMPARISON OF SILICA-, C_{18}-, AND NH_2-HPLC COLUMNS FOR THE SEPARATION OF NEUTRAL STEROID SAPONINS FROM *DIOSCOREA* PLANTS

Cheng-Ji Xu and Jiann-Tsyn Lin*
Plant Physiology and Chemistry Research Unit
Western Regional Research Center
Agricultural Research Service
U.S. Department of Agriculture
Albany, California 94710

ABSTRACT

Six neutral steroid saponins including dioscin and gracillin from the tubers of Dioscorea plants and their peracetates were chromatographed by silica-, C_{18}-, and NH_2-column high-performance liquid chromatography (HPLC). The eluent containing water was used in the silica-column HPLC for the separation of these saponins. These HPLC systems complement each other and allow separation of the saponins. The number of carbohydrate units in the saponin molecule plays the most important role for the separations. The more carbohydrate units in the molecule, the more polar is the saponin or its peracetate. Separation of saponins containing the same number of carbohydrate units can be improved by using their peracetates.

INTRODUCTION

Saponins are widely distributed in the plant kingdom and have been regarded as the significant active components of many medici-

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nal herbs[1]. The saponin extract from the tubers of Dioscorea nipponica Makino has been used in China in the form of tablets to treat coronary arteriosclerosis[2]. Some neutral steroid saponins from the tubers of Dioscorea colletti Hook. f. have been isolated and identified[3]. In this communication we report the separation of these saponins and their peracetates using three different HPLC columns. These HPLC systems may be used to separate and purify unknown saponins which are responsible for the pharmacological activity of the saponin extract from the tubers of Dioscorea plants. The elucidation of the structures of these active saponins may also become possible.

A few HPLC separations of saponins have been reported, mostly the HPLC of glycoalkaloids (basic steroid saponins). Separations of three potato glycoalkaloids (α-chaconine, β-chaconine and α-solanine) have been achieved using three different columns: μBondapak C_{18}, μBondapak NH_2, and a carbohydrate analysis column[4]. A Zorbax semi-preparative NH_2 column has been used to separate a crude mixture of the potato glycoalkaloids, α-chaconine, α-solanine, commersonine and demissine[5]. A carbohydrate analysis column also has been used to analyze the contents of α-chaconine and α-solanine in potato tubers[6]. The separation and analysis of solasodine glycosides from the leaves of solanum plants has been reported using μBondapak C_{18} and a carbohydrate analysis column[7]. The HPLC of neutral steroid saponins, avenacosides A, B, C, and D, from Avena sativa has also been reported using Lichrosorb RP8 column[8]. However, the HPLC of neutral steroid saponins from Dioscorea plants has not been reported.

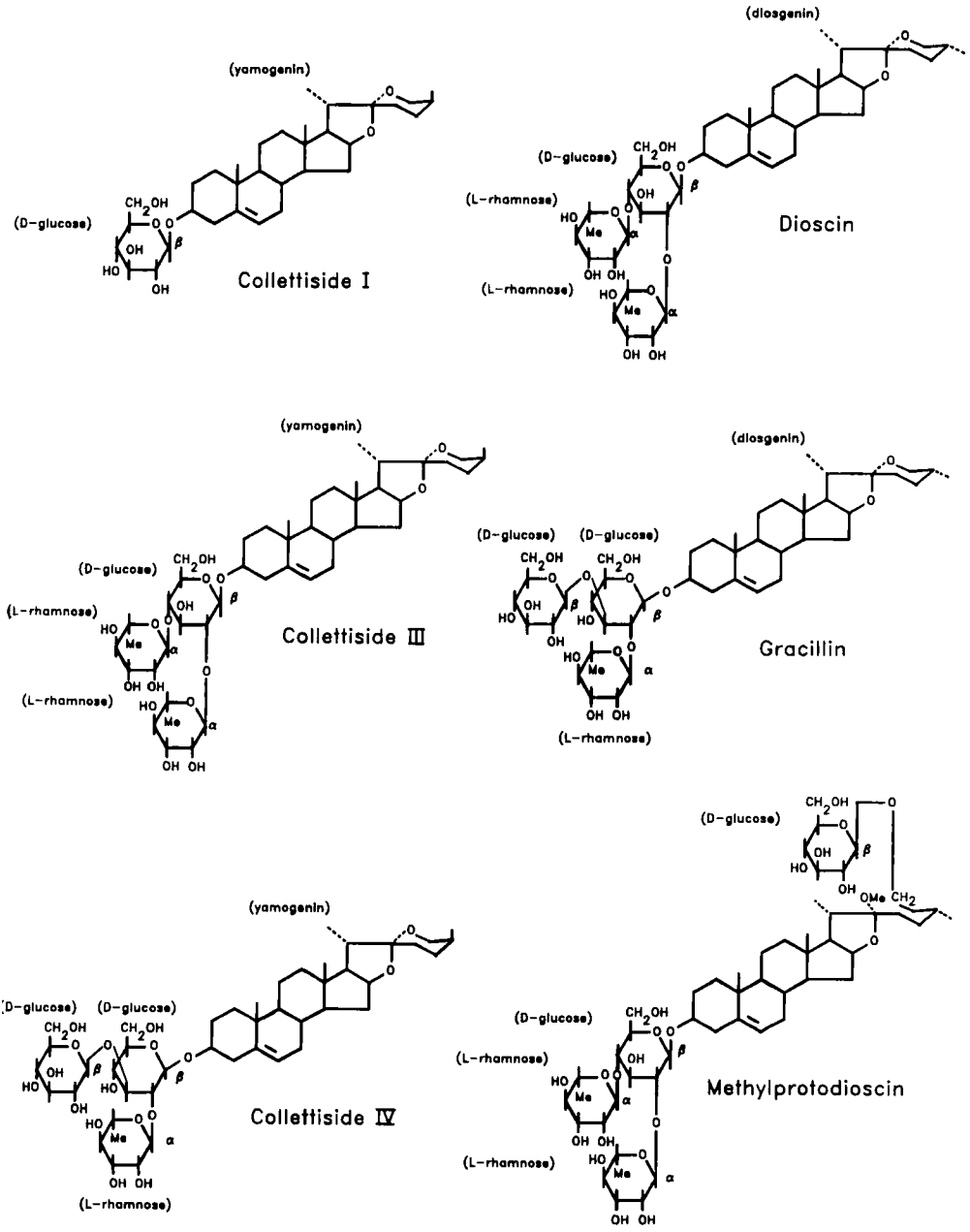

FIGURE 1. Structures of some neutral steroid saponins from Dioscorea plants.

METHODS

The HPLC system consisted of a pump (Model 110A, Beckman, Berkeley, California), a sample injector (Model 7125, Rheodyne, Cotati, California) with a loop volume of 1 ml, a variable wavelength UV-VIS detector (Model 155-30, Beckman) and a recorder (Model 385, Linear, Irvine, California). The prepacked HPLC columns used were Spherisorb S-5-W (silica, Alltech, Deerfield, Illinois), Ultrasphere ODS (C_{18}, Beckman) and Spherisorb S-5-NH_2 (Alltech). All of the columns were 25 cm x 0.46 cm with particle size of 5 μ. Chromatographic conditions are given in the legends of Figures 2 and 3. Saponin peracetates were prepared by acetylation of saponins with pyridine and acetic anhydride (Acetylation Kit, Applied Science Laboratories, Deerfield, Illinois).

RESULTS AND DISCUSSION

The results are summarized in Table I. Retention times shown never varied by more than ± 5%. The six steroid saponins are shown in the order of elution of saponins in silica-column HPLC (System 1). Silica-column HPLC has not previously been reported as separating saponins. The eluent of hexane-ethanol alone in silica-column HPLC produced peaks of saponins which were very broad, and no separation of these saponins was possible. Addition of water to the eluent (System 1) made the peaks very sharp and resolution possible.

The number of carbohydrate units present in the saponin molecule plays the most important role in the separation of both saponins and their peracetates in silica-, C_{18}-, and NH_2-column HPLC. Collettiside I containing one carbohydrate unit is the least polar

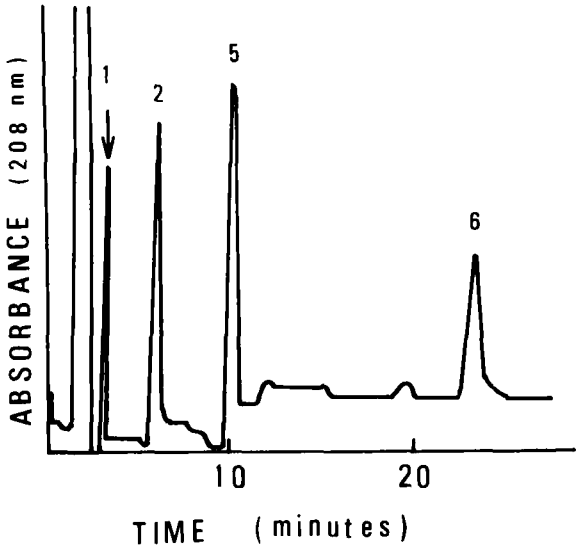

FIGURE 2. NH_2-column chromatogram of steroid saponins. Between 5 μg (collettiside I) and 50 μg (methylprotodioscin) of saponins dissolved in 200 μl of the eluent were chromatographed on a column of Spherisorb S-5-NH_2, 25 cm x 0.46 cm. Eluent, acetonitrile-water (85:15); flow rate, 2 ml/min; pressure, 3000 psi; UV detector, 208 nm, range 0.1; recorder, speed 12 cm/h, span 10 mV. Compound numbers are the same as in Table I.

TABLE I

HPLC Retention Times (min.) of Steroid Saponins
Free saponins were chromatographed in Systems 1, 2 and 3, peracetates in all other systems. System 1, silica column, hexane-ethanol-water (8:2:0.5); System 2, C_{18}-column, methanol-water (78:22); System 3, NH_2-column, acetonitrile-water (85:15), see Fig. 2; System 4, silica column, hexane-ethanol (95:5), see Fig. 3; System 5, C_{18}-column, methanol-water, (9:1); System 6, NH_2-column, acetonitrile-water (98:2). Flow rate, 2 ml/min, except that of System 2 was 1 ml/min. Capacity factors, $k' = t - t_o/t_o$, $t_o = 1.25$ (Systems 1 and 4), $t_o = 1.80$ (System 2), $t_o = 0.90$ (System 5), $t_o = 0.70$ (Systems 3 and 6).

No.	Compound	Systems 1	2	3	4	5	6
1	Collettiside I	5.25	15.75	3.25	4.75	12.25	5.25
2	Collettiside III	9.25	11.25	6	8.75	8.5	10.25
3	Collettiside IV	9.25	11.25	6	5.75	8.5	9.5
4	Dioscin	9.25	11.25	6	8.25	8.5	10.25
5	Gracillin	12.25	11.25	10.25	5.75	11.25	7.25
6	Methlyprotodioscin	44	2.25	23.5	10.25	4.25	15

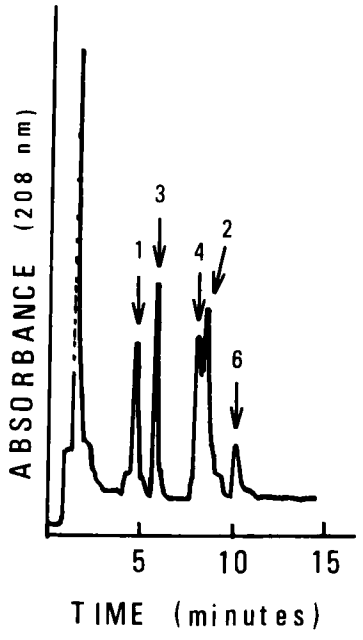

FIGURE 3. Silica-column chromatogram of steroid saponin peracetates. Conditions as in Fig. 2 except a column of Spherisorb S-5-W, 25 cm x 0.46 cm, and an eluent of hexane-ethanol (95:5) were used. Compound numbers are the same as in Table I.

compound among these six saponins (Table I). Methylprotodioscin containing four carbohydrate units is the most polar compound. The other compounds contain three carbohydrate units. The more carbohydrate units present in the molecule result in a more polar saponin or peracetate. The relationship of polarity to the number of carbohydrate units in the molecule is similar to that in previous reports[4,7] of the HPLC of glycoalkaloids using C_{18}- and NH_2-columns. As shown in Table I, only saponins differing by a carbohydrate unit can be resolved in C_{18}-column HPLC. This behavior is also similar to that of glycoalkaloids in previous reports[4,7].

Silica-column HPLC and NH_2-column HPLC are both normal-phase, while C_{18}-column HPLC is reversed-phase. We have recently reported that the 25-epimers of the sapogenins, diosgenin and yamogenin, can not be separated well by either the silica column (eluent, hexane-ethanol, 98:2) or the C_{18}-column (eluent, methanol-water, 85:15) HPLC[9]. In this report as shown in Table I (Systems 1, 2 and 3), 25-epimers of saponins, collettiside III and dioscin, can not be separated by any of the three columns, while 25-epimers of saponins, collettiside IV and gracillin, can be separated by the normal-phase columns (Systems 1 and 3) but not by the reversed-phase column (System 2). The eluents of normal-phase HPLC of saponins contained water (Systems 1 and 3), while the eluent for the separation of sapogenins by silica column was hexane-ethanol (98:2). Peracetates of collettiside III and dioscin can be partially separated by the silica column (System 4) but not by the other two columns (Systems 5 and 6). Peracetates of collettiside IV and gracillin can be separated by both C_{18}- and NH_2-columns (Systems 5 and 6), but not by the silica column (System 4).

Collettisides III and IV can not be separated by any of the three columns (System 1, 2 and 3) as shown in Table I; however, their peracetates can be separated by normal-phase HPLC (Systems 4 and 6). Dioscin and gracillin can be separated by both of the normal-phase HPLC (Systems 1 and 3). Their peracetates can be separated by all of the three columns (Systems 4, 5 and 6). Dioscin is less polar than gracillin in normal-phase HPLC Systems (1 and 3), while dioscin peracetate is more polar than gracillin perace-

tates in all of the three column HPLC Systems (4, 5 and 6). Separations of saponins containing the same number of carbohydrate units can be improved by using their peracetates. These six HPLC systems as shown in Table I complement each other and allow the separation and identification of these steroid saponins.

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