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Two-Dimensional TLC Analysis of Ginsenosides from Root of Dwarf Ginseng (*Panax trifolius* L.) Araliaceae

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Received May 15, 1980, from the Department of Biological Sciences, Philadelphia College of Pharmacy and Science, Philadelphia, PA 19104. Accepted for publication July 3, 1980.

Abstract □ A procedure is reported for the two-dimensional TLC separation of ginsenosides from the root of *Panax trifolius* L. The solvent systems and spray reagents described are useful for the identification of ginsenosides in various species of *Panax*. The results of the separation and identification with controls of at least four ginsenosides from the root of *P. trifolius* L. are reported. The total percentage of ginsenosides was 0.0061%.

Keyphrases □ Ginsenosides—two-dimensional TLC analysis from root of dwarf ginseng □ *Panax trifolius* L.—two-dimensional TLC analysis of ginsenosides from root □ Ginseng, dwarf—two-dimensional TLC analysis of ginsenosides from root

Dwarf ginseng¹ is a member of the ginseng family (Araliaceae) and is distributed from southern Canada to the northern United States (1). The plant is small and delicate and has a whorl of three stalked leaves, each of which is divided into three stalkless leaflets. The plant has a small round umbel of white flowers, which develop into greenish-yellow fruits, and a round tuberous root, which grows deep in the ground.

This report describes the usefulness of two-dimensional TLC for the semimicro separation of the ginsenosides isolated from the root of dwarf ginseng (*Panax trifolius* L.).

EXPERIMENTAL

Plant Material—Specimens of wild dwarf ginseng (*P. trifolius* L.) were collected on May 4, 1979, from the Tyler Arboretum, Delaware County, Pa.

Extraction Procedure—Ground, freeze-dried roots (18.6 g) were extracted with chloroform (150 ml) to remove pigments and lipids. The marc was air dried and extracted again with methanol (150 ml). Methanol-washed silica gel (10.0 g) was mixed well with the methanolic extract and filtered to remove impurities (2). The filtrate was combined with the

methanolic silica gel washings and concentrated to 5 ml. Ten milliliters of water was added to 50 ml of the methanolic extract and then reextracted three times with water-saturated 1-butanol (60 ml total). The three butanol layers were combined and concentrated to yield the crude saponin extract (20 mg), and this extract was examined by two-dimensional TLC.

Two-Dimensional TLC—*Adsorbent*—Precoated silica gel 60 F-254 silanized TLC plates² were used (20 × 20 cm, 250-μm layer).

Eluent Systems—Solvents were analytical grade and were used as received³. Eluent A was chloroform-methanol-ethyl acetate-butanol-water (4:4:8:1:2, lower phase). Eluent B was chloroform-butanol-methanol-water (4:8:3:4, lower phase). Eluent C was chloroform-methanol-water (13:7:2, lower phase).

Detection Reagent—A solution of 1 ml of *p*-anisaldehyde and 1 ml of concentrated sulfuric acid diluted to 100 ml with methanol was prepared fresh daily. Plates were heated at 130° for 15 min following spraying (3).

Standard Solutions—Standard solutions consisted of ginsenosides⁴ Ro, Rb₁, Rb₂, Rc, Rd, Re, Rf, Rg₁, and Rg₂ dissolved in methanol at a concentration of 1 mg/ml. All stock solutions were kept refrigerated (3).

Procedure—The concentrated crude saponins were dissolved in methanol (1 ml), and 20 μl of this solution was spotted on the bottom left corner of the plates 2.0 cm from each side. The standard solutions of 5 μl of each control ginsenoside were spotted separately 0.8 cm from each other on the bottom right and upper left, exactly 10.0 cm from the crude saponin spot. Two-dimensional TLC plates were prepared as shown in Fig. 1.

The plates were developed separately in one direction in Eluents A and B for 10 cm. After air drying for 10 min, the plates were run in the second direction using Eluent C perpendicular to the first direction for a distance of 10 cm. The *R_f* values were calculated on the basis of a solvent front of 10.0 cm above the origin, and the relative *R_f* values were calculated with the ginsenoside Rf as the standard.

Quantitative Analysis—Quantitation of the saponins of *P. trifolius* L. was performed using the spectrodensitometric procedure described previously (3). A 250-mg portion of the root was extracted, and 3.5 ml of the final 1-butanol solution was evaporated to dryness. The residue was

² Catalog No. 5601, EM Laboratories, Elmsford, NY 10523.

³ Fisher Scientific Co., Fair Lawn, NJ 07410.

⁴ Nine standard ginsenosides were obtained from Dr. J. Shoji, Showa University, Tokyo, Japan.

¹ This is the first report of continuing research on *Panax trifolius* L. All plant parts of dwarf ginseng are currently being studied in detail.

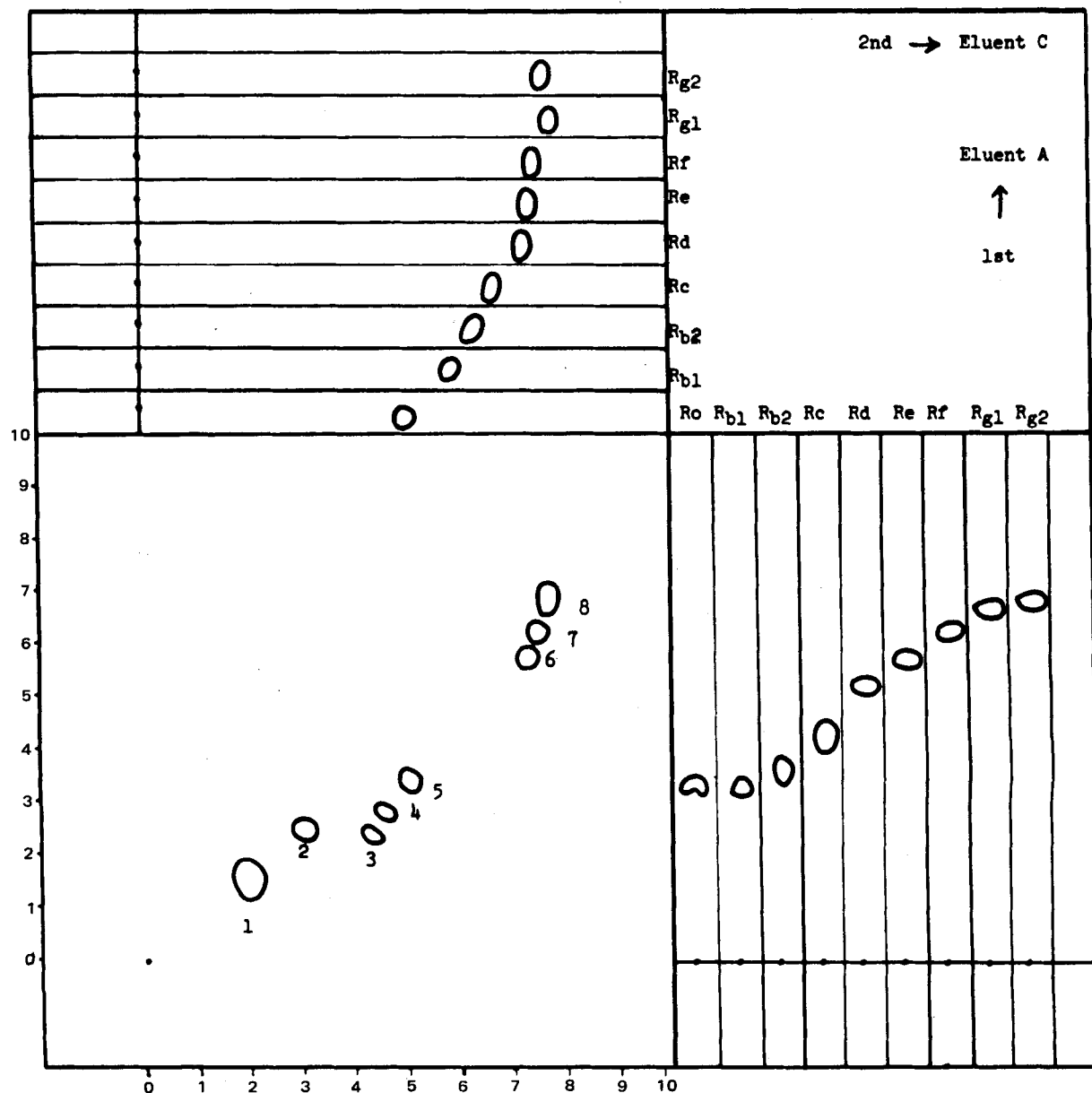


Figure 1—Chromatogram of the two-dimensional identification for ginsenosides from the root of *P. trifolius* L. using Eluents A and C.

Table I—The R_f and RR_f Values and Color Reactions of the Reference and Examined Ginsenosides from the Root of *P. trifolius* L.

Ginsenoside ^a	Eluent A		Eluent B		Eluent C		Color Reaction ^c
	R_f	RR_f^b	R_f	RR_f^b	R_f	RR_f^b	
Standards							
Ro	0.34	0.55	0.63	0.74	0.50	0.68	B
Rb ₁	0.33	0.53	0.63	0.74	0.59	0.80	V
Rb ₂	0.37	0.60	0.64	0.75	0.63	0.85	V
Rc	0.42	0.68	0.69	0.81	0.67	0.91	V
Rd	0.52	0.84	0.78	0.92	0.72	0.97	V
Re	0.57	0.92	0.88	1.04	0.73	0.99	G
Rf	0.62	1.00	0.85	1.00	0.74	1.00	G
Rg ₁	0.66	1.06	0.98	1.15	0.77	1.04	G
Rg ₂	0.68	1.10	0.92	1.08	0.76	1.03	G
<i>P. trifolius</i>							
1	0.15	0.24	0.24	0.28	0.20	0.27	G
2	0.25	0.40	0.37	0.44	0.30	0.41	G
3	0.24	0.39	0.69	0.81	0.43	0.58	G
4	0.28	0.45	0.73	0.86	0.46	0.62	G
5 (Ro)	0.34	0.55	0.63	0.74	0.50	0.68	B
6 (Re)	0.57	0.92	0.88	1.04	0.73	0.99	G
7 (Rf)	0.62	1.00	0.85	1.00	0.74	1.00	G
8 (Rg ₂)	0.68	1.10	0.92	1.08	0.76	1.03	G

^a The numbers refer to the numbers given to each spot in Fig. 1. ^b Relative R_f values with the ginsenoside Rf as the standard. ^c B = blue, V = violet, and G = green.

redissolved in 0.2 ml of 1-butanol, and 16 μ l was applied to alternate lanes of the TLC plates⁵, developed, and assayed by spectrodensitometry.

RESULTS AND DISCUSSION

Ginseng is one of the best-known Chinese drugs and has been claimed to be useful in increasing lifespan as well as being a cure-all. The ginsenosides, abbreviated as Rx, are considered as the characteristic and active principles that account for the major biological activities of ginseng (4).

Chemically, all ginsenosides are dammarane-type triterpenoid saponins, except for Ro, which is an oleanane type. The dammarane-type compounds are classified further into two groups possessing either 20-S-protopanaxadiol or 20-S-protopanaxatriol as the sapogenin.

In this study, eight ginsenosides were found in the root of *P. trifolius* L. Of these compounds, Ro, Re, Rf, and Rg₂ were identified with nine of the controls. Combinations of relative R_f and RR_f (R_f values with ginsenoside Rf as the standard) values and the specific color reactions after spraying with detection reagent facilitated identification of the ginsenosides.

Table I shows those ginsenosides (Rb₁, Rb₂, Rc, and Rd) with 20-S-protopanaxadiol as the sapogenin. These compounds show a violet color after spraying with the detection reagent. Those with 20-S-protopanaxatriol as the sapogenin (Re, Rf, Rg₁, and Rg₂) show a green color, while the oleanane-type triterpenoid saponin (Ro) yields a blue color. The ginsenosides denoted as 1-4 in Fig. 1 all were green after spraying, indicating that they are dammarane-type triterpenoid saponins with 20-S-protopanaxatriol as the sapogenin.

In this two-dimensional TLC procedure, identifications depend on the R_f and RR_f values and on color. These parameters provide a great advantage for the detection of ginsenosides, not only in the dwarf ginseng but also in other species of *Panax*.

Eight separate spots were observed by quantitative analysis, but the darkness of the spots, while sufficient for quantitation, was not sufficient for determination of the color class by this procedure. The results confirmed the presence of ginsenosides Ro, Re, Rf, and Rg₂ (Table II).

The identities of the remaining four saponins are unknown. The total saponin content of *P. trifolius* (including unknowns) was found to be the

Table II—Individual and Total Saponin Content of *P. trifolius* Root^a

Ginsenoside ^b	Individual Saponin, %	R_f^c	RR_f^d
1 (Ro)	0.0004	0.10	0.18
2	0.0006	0.25	0.47
3	0.0008	0.32	0.56
4	0.0012	0.38	0.67
5 (Re)	0.0005	0.47	0.82
6	0.0005	0.51	0.89
7 (Rf)	0.0008	0.57	1.00
8 (Rg ₂)	0.0008	0.63	1.11
9 (Sapogenins)	0.0011	0.71	1.25

^a Total saponins = 0.0061%. ^b Due to a different procedure and solvent system, the numbering of the ginsenosides does not necessarily correspond to that in Table I. ^c The solvent system was methanol-chloroform-1-butanol (1:1:1). ^d Relative R_f values with ginsenoside Rf as standard.

lowest (0.0061%) of any roots belonging to this genus that have been examined in these laboratories.

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ACKNOWLEDGMENTS

The authors thank Dr. J. Shoji, Showa University, Tokyo, Japan, for authentic samples of ginsenosides, Mr. Robert Montgomery and Mr. John Beutler, Tyler Arboretum, Delaware County, Pa., for dwarf ginseng plant materials, and Mr. Joseph Betz, Philadelphia College of Pharmacy and Science, Philadelphia, Pa., for the spectrodensitometric analysis of the ginseng extract. They also thank Bristol Myers, Industrial Division at Syracuse, N.Y., for use of their facilities.

⁵ Analtech Co., Newark, DE 19711.

Selenium-Sulfur Analogs III: Synthesis and Biodistribution of Two ⁷⁵Se-Labeled 4-Substituted-1,2,3-selenadiazoles

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Received March 31, 1980, from the Departments of Medicinal Chemistry and Pharmacology, College of Pharmacy and Allied Health Professions, Northeastern University, and Department of Radiology, Joint Program in Nuclear Medicine, Harvard Medical School, Boston, MA 02115. Accepted for publication July 11, 1980.

Abstract □ The ⁷⁵Se-labeled 4-substituted-1,2,3-selenadiazole analogs of two drugs that inhibit the adrenocorticosteroid 11 β - and 17 α -hydroxylase enzymes were prepared by the [⁷⁵Se]selenious acid oxidation of the appropriate methyl ketone semicarbazones. The concentration of the radiolabeled compounds in the adrenal glands of rats over a 0.25-24-hr period was determined and compared with that in the blood, liver, and kidneys. The concentration in the adrenal glands and the target to nontarget ratios were much lower than those reported for other adre-

nocorticosteroid inhibitors. Therefore, these 1,2,3-selenadiazole agents do not have potential as adrenocorticosteroid imaging agents.

Keyphrases □ 1,2,3-Selenadiazoles—synthesis and biodistribution of radiolabeled compounds as potential adrenocorticosteroid imaging agents
□ Imaging agents, potential—radiolabeled 1,2,3-selenadiazoles, synthesis and biodistribution

One current approach to the development of adrenal cortex imaging agents employs radiolabeled analogs of drugs that inhibit adrenocorticosteroid biosynthesis (1-3). Although Beierwaltes *et al.* (1-3) reported the *in vivo* vi-

sualization of the canine adrenal cortex with ¹³¹I-labeled aminophenylethylamine, their studies suggested that radiolabeled derivatives of the 11 β - and 17 α -hydroxylase enzyme inhibitors might be more effective. Therefore, an