redissolved in 0.2 ml of 1-butanol, and 16 μl was applied to alternate lanes of the TLC plates[5], 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 Rg_2 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 (Rb_1, Rb_2, 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, Rg_1, and Rg_2) 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 Rg_2 (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 lowest (0.0061%) of any roots belonging to this genus that have been examined in these laboratories.

[5] Analtech Co., Newark, DE 19711.

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.

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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.

Selenium–Sulfur Analogs III: Synthesis and Biodistribution of Two ^{75}Se-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 ^{75}Se-labeled 4-substituted-1,2,3-selenadiazole analogs of two drugs that inhibit the adrenocorticosteroid 11β- and 17α-hydroxylase enzymes were prepared by the [^{75}Se]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 adrenocorticosteroid 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* visualization of the canine adrenal cortex with ^{131}I-labeled aminophenylethylamine, their studies suggested that radiolabeled derivatives of the 11β- and 17α-hydroxylase enzyme inhibitors might be more effective. Therefore, an

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Table I—Percent of Injected Dose per Organ of [^{75}Se]-I*b* following Intravenous Administration

Organ	15 min	2 hr	6 hr	24 hr
Liver	37.62	36.72	23.62	14.83
	31.46–52.45[a]	26.23–46.70	19.73–27.40	12.85–16.85
Spleen	0.29	0.31	0.57	0.56
	0.21–0.40	0.26–0.37	0.39–0.80	0.48–0.63
Lungs	7.55	4.99	2.70	1.84
	6.06–10.84	4.22–6.40	2.45–2.90	1.61–2.02
Heart	0.24	0.23	0.23	0.17
	0.14–0.29	0.20–0.26	0.20–0.26	0.13–0.21
Small intestine	13.43	17.20	5.74	2.92
	10.81–14.80	14.78–18.64	3.98–7.74	2.64–3.14
Large intestine	1.46	3.30	12.59	4.43
	0.88–1.83	1.28–7.67	9.52–15.13	2.54–6.97
Kidneys	2.02	4.60	2.86	2.47
	1.91–2.16	4.30–4.86	2.64–4.19	1.94–3.21
Muscle	14.20	10.73	17.80	9.45
	10.43–23.41	6.86–15.09	8.36–23.31	8.22–11.31
Fat and fur	15.59	7.90	9.25	5.31
	9.97–23.35	6.70–8.68	5.08–11.76	4.09–7.16
Bone	4.61	3.90	4.58	4.61
	2.81–6.92	2.68–6.86	3.97–5.45	3.98–5.82
Blood	8.42	9.65	8.10	6.81
	6.63–13.03	6.77–13.08	6.66–8.86	5.32–8.42
Adrenal	0.03	0.03	0.02	0.02
	0.02–0.03	0.02–0.04	0.01–0.03	0.01–0.03

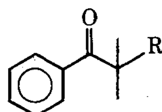
[a] Mean and range for four rats.

attempt was made to utilize the structural similarity between pyridine and the 1,2,3-selenadiazole groups and the properties of selenium 75.

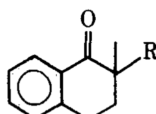
The 1,2,3-selenadiazolyl (I*b* and II*b*) and 1,2,3-thiadiazolyl (I*c* and II*c*) analogs of two drugs, 2-(3-pyridyl)-2-methylpropiophenone (I*a*) and 2-(3-pyridyl)-2-methyl-1-tetralone (II*a*), that inhibit the 11β- and 17α-hydroxylase enzymes, respectively (4, 5), were prepared. In this study, I*b* and II*b* were radiolabeled and their biodistribution was determined in normal rats to evaluate their potential as adrenal cortex imaging agents. These analogs were selected rather than the thiadiazoles because they are synthesized easily and selenium 75 is readily available.

EXPERIMENTAL

General Method for Preparation of ^{75}Se-Labeled 4-Substituted-1,2,3-selenadiazoles (I*b* and II*b*)—Five millicuries (0.5 ml) of [^{75}Se]selenious acid in 0.5 *M* HCl was added to a solution composed of 25 μmoles of the semicarbazone, 26 μmoles of selenium dioxide, and 0.5 ml of acetic acid (Schemes I and II). The mixture was heated at 60° for 1 hr, cooled, diluted with 2 ml of water, and extracted with ethyl acetate. The ethyl acetate layer was evaporated, and the residue was purified by alumina column chromatography with ethyl acetate as the eluent to yield the desired product (7.5 μmoles, 30%). Thin-layer radiochromatography on alumina or silica gel using ethyl acetate as the eluent indicated the presence of a single radioactive component (>98%), which comigrated



I*a*: R = 3-pyridyl
I*b*: R = 4-(1,2,3-selenadiazolyl)
I*c*: R = 4-(1,2,3-thiadiazolyl)



II*a*: R = 3-pyridyl
II*b*: R = 4-(1,2,3-selenadiazolyl)
II*c*: R = 4-(1,2,3-thiadiazolyl)

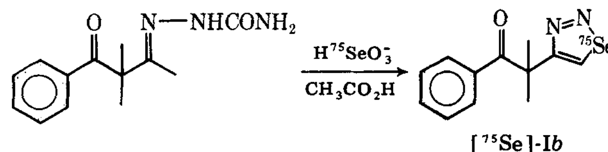
with an authentic sample prepared using the same procedure but without the [^{75}Se]selenious acid and was characterized by IR and NMR spectroscopy[1]. The radiolabeled product was dissolved in 2.0 ml of propylene glycol. Because of the chemical instability of the 1,2,3-selenadiazoles to heat and light, it was stored at 4° in the dark for subsequent biodistribution studies.

Animal Studies—The biodistribution of the radiolabeled compounds (I*b* and II*b*) was determined in outbred Wistar or Sprague–Dawley rats of either sex (150–200 g). The rats were lightly anesthetized with ether, and a propylene glycol solution of the radiopharmaceutical (0.1 ml, 7.5 μCi) was injected into the femoral vein. Groups of three or four animals subsequently were sacrificed by ether asphyxiation at 0.25, 2.6, and 24 hr after administration.

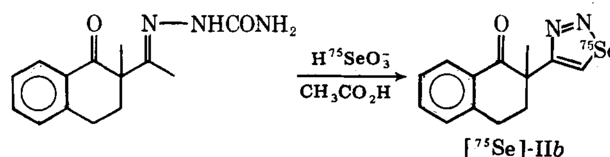
Blood was obtained from a vein in the thoracic cavity. The organs of interest were carefully excised, weighed, and counted in a sodium iodide (Tl) γ-well scintillation counter. The data were converted to the percent of injected dose per organ. The ratios of the agent concentration (percent of injected dose per gram) in the adrenal cortex (target organ) compared with that in the liver, kidneys, and blood (nontarget organs) were calculated.

RESULTS AND DISCUSSION

Chemistry—The [^{75}Se]selenious acid oxidation of the appropriate methyl ketone semicarbazone (6–8) provided the desired ^{75}Se-labeled 4-substituted-1,2,3-selenadiazoles (I*b* and II*b*). Extraction with ethyl



Scheme I



Scheme II

[1] The analytical data were: I*b*, IR (KBr): 1670 cm^{-1}; NMR ($CDCl_3$ + tetramethylsilane): δ 1.88 (6H) and 9.00 (1H); and II*b*, IR (neat): 1680 cm^{-1}; NMR ($CDCl_3$ + tetramethylsilane): δ 1.76 (3H) and 8.67 (1H).

Table II—Percent of Injected Dose per Organ of [^{75}Se]-II*b* following Intravenous Administration

Organ	15 min	2 hr	6 hr	24 hr
Liver	39.14	27.90	18.78	10.31
	35.54–43.17[a]	25.71–32.17	16.06–22.36	7.22–12.59
Spleen	0.27	0.30	0.40	0.35
	0.20–0.32	0.27–0.37	0.27–0.50	0.21–0.53
Lungs	5.57	3.03	3.25	2.12
	5.07–6.36	2.54–3.40	2.50–3.72	1.67–2.37
Heart	0.41	0.26	0.18	0.16
	0.31–0.60	0.18–0.36	0.16–0.19	0.14–0.18
Small intestine	18.31	17.04	8.30	3.88
	14.54–22.70	15.28–19.20	7.73–9.35	3.00–4.36
Large intestine	2.76	2.22	15.29	2.64
	2.32–3.13	1.47–2.85	14.06–16.65	2.16–3.49
Kidneys	3.26	3.39	3.87	2.11
	2.54–3.76	2.52–4.37	3.68–4.16	1.95–2.30
Muscle	9.49	15.19	16.86	9.32
	6.72–11.40	9.53–18.91	12.34–23.32	6.48–14.38
Fat and fur	12.04	12.91	13.05	7.99
	10.26–15.06	7.66–15.87	9.60–15.22	7.41–8.83
Bone	2.63	4.66	8.97	5.23
	1.87–3.07	3.03–7.21	7.51–9.98	3.36–6.24
Blood	6.97	4.17	6.68	5.23
	6.76–7.15	3.84–4.41	5.15–8.60	4.00–6.97
Adrenal	0.04	0.03	0.04	0.03
	0.03–0.04	0.02–0.04	0.03–0.06	0.01–0.07

[a] Mean and range for three rats.

Table III—Adrenal Uptake and Target to Nontarget Ratios for [^{75}Se]-I*b* and [^{75}Se]-II*b*

	Adrenal Uptake, % of injected dose/g		Target to Nontarget Ratios					
			Adrenal/Kidney		Adrenal/Liver		Adrenal/Blood	
Time	I*b*	II*b*	I*b*	II*b*	I*b*	II*b*	I*b*	II*b*
15 min	1.07	0.62	1.07	0.59	0.30	0.25	2.10	2.20
2 hr	0.65	1.24	0.27	0.45	0.19	0.23	1.25	2.31
6 hr	0.57	1.50	0.40	0.46	0.26	0.36	1.08	1.84
24 hr	0.75	1.09	0.47	0.62	0.37	0.56	1.42	1.69

acetate followed by column chromatographic separation gave the products, isolated in 10–30% radiochemical yield, with >98% radiochemical purity. The specific activity of the material varied from 60 to 220 mCi/mmole. The ^{75}Se-labeled selenadiazoles were dissolved in propylene glycol and stored in the dark at 4° for use in the biodistribution study.

Animal Studies—The deposition and excretion patterns of [^{75}Se]-I*b* and [^{75}Se]-II*b* were similar (Tables I and II). For each agent, the liver was the primary site of localization at all time periods examined with significant percentages in the muscle, fat, and fur. Blood levels remained high over the 24-hr period, and only 50% of the label was excreted in that time. Unfortunately, the adrenal uptake of I*b* was 0.57–1.07% of the injected dose/g and that of II*b* was 0.56–1.51% of the injected dose/g, and these values are much lower than those reported for other labeled adrenocortical enzyme inhibitors (1–3). The adrenal to liver and adrenal to kidney values (Table III) for the ^{75}Se-labeled compounds both were <1, whereas the corresponding values for the other labeled inhibitors were 2–11 and 3–20. The lack of localization and selectivity and the slow clearance of the label from the body indicate that the ^{75}Se-labeled 4-substituted-1,2,3-selenadiazoles (I*b* and II*b*) do not possess the characteristics necessary for adrenal imaging agents.

Two factors may contribute to the failure of these compounds to localize adequately in the adrenal cortex. First, the compounds may not be sufficiently selective for the adrenal cytochrome P-450-linked hydroxylase enzymes relative to the hepatic cytochrome P-450-linked metabolic enzymes. Second, metabolism of the 1,2,3-selenadiazoles by the liver may result in the deposition in the organ of selenium 75, which is only slowly excreted.

The results of this study suggest that subsequent work should be directed toward the synthesis of more chemically stable selenium-containing compounds that possess greater selectivity for the adrenal cortex.

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ACKNOWLEDGMENTS

Supported in part by the Jane Coffin Child Memorial Fund and by Department of Health, Education, and Welfare Grant RR 07143, National Institutes of Health Grants HL 17739 and CA 19898, and Department of Energy Contract EY-76-S-4115.