Acknowledgment. This work was supported by the National Science Foundation, Undergraduate Science Education Program Grant GY-8956.

References

(1) H. H. Meyer, *Arch. Exp. Pathol. Pharmacol.*, **46**, 338 (1901).
(2) E. Overton, *Pfluegers Arch. Gesamte Physiol. Menschen Tiere*, **92**, 115 (1901).
(3) K. W. Miller, D. M. Patton, and E. B. Smith, *Nature (London)*, **206**, 574 (1965); *Brit. J. Anaesth.*, **39**, 910 (1967).
(4) L. J. Saidman, E. I. Eger, E. S. Munson, A. A. Babad, and M. Muallem, *Anesthesiology*, **28**, 994 (1967).
(5) H. J. Lowe and K. Hagler in "CIBA Symposium on Gas Chromatography," R. Porter, Ed., J. A. Churchill Ltd., London, 1969, pp 86–103.
(6) S. L. Miller, E. I. Eger, and C. Lundgren, *Nature (London)*, **221**, 468 (1961).
(7) L. Pauling, *Science*, **134**, 15 (1961).
(8) S. L. Miller, *Proc. Nat. Acad. Sci. U. S.*, **47**, 1515 (1961).
(9) E. I. Eger, C. Lundgren, S. L. Miller, and W. C. Stevens, *Anesthesiology*, **30**, 129 (1969).
(10) A. L. Hodgkin, "The Conduction of the Nervous Impulse," Charles C Thomas, Springfield, Ill., 1964.
(11) I. Tasaki, *Amer. J. Physiol.*, **127**, 211 (1939); "Nervous Transmission," Charles C Thomas, Springfield, Ill., 1953, pp 90–93.
(12) E. B. Wright, *Amer. J. Physiol.*, **148**, 174 (1947).
(13) F. G. Carpenter, *ibid.*, **178**, 505 (1954).

Potential Organ- or Tumor-Imaging Agents. 13.† 19-Radioiodinated Sterols‡

R. E. Counsell,* P. G. Kulkarni, P. Afiatpour, and V. V. Ranade

Laboratory of Medicinal Chemistry, College of Pharmacy, The University of Michigan, Ann Arbor, Michigan 48104.
Received December 21, 1972

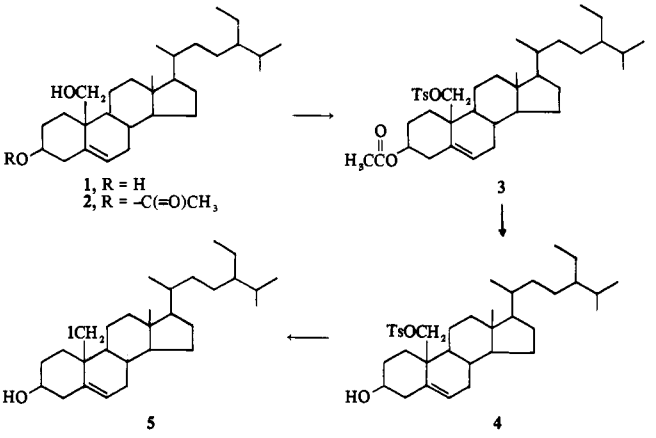
Over the past several years an effort has been made in our laboratory to develop a radiopharmaceutical suitable for photoscanning the adrenal gland and associated tumors. Recently, we reported that 19-iodocholesterol-^{125}I selectively concentrated in the adrenal glands of dogs in a manner similar to cholesterol-4-^{14}C and resulted in the first *in vivo* visualization of the dog adrenals.[2] Although the ^{125}I-labeled sterol was suitable for small animal studies, subsequent trials in man have required the more penetrating γ-radiation afforded by the ^{131}I-labeled sterol. In this form, 19-iodocholesterol has demonstrated its utility in the clinical diagnosis of unilateral adrenocortical adenoma,[3] primary aldosterone adenoma,[4] and Cushing's syndrome.[5,6]

As a follow-up to these studies, we became interested in ascertaining the structural requirements essential for optimal adrenal uptake. Nagai, *et al.*,[7] had previously noted that tritiation of stigmasterol produced a labeled preparation§ which in rats showed an adrenal/liver mean concentration ratio of 22.07 ± 5.29 at 48 hr. This value was almost double that obtained in our laboratory (12.33 ± 0.89) for cholesterol-4-^{14}C under similar conditions. Accordingly, these results prompted us to synthesize 19-iodo-β-sitosterol-^{125}I and to evaluate its ability to selectively localize in adrenal cortical tissue.

A search of the literature revealed that 19-hydroxysitosterol (**1**) and the corresponding 3-monoacetate **2** had been prepared[8] from β-sitosterol *via* the method of Bowers, *et al.*[9] The preparation of **2** by this route was duplicated in our laboratory and served as the starting material for the preparation of 19-iodo-β-sitosterol (**5**).

Treatment of **2** with *p*-toluenesulfonyl chloride in pyridine afforded the desired tosylate ester **3**. Selective hydrolysis of the 3-acetate function gave **4** which upon subsequent treatment with sodium iodide in 2-propanol afforded 19-iodo-β-sitosterol (**5**) (Scheme I). Radioiodination was readily

Scheme I



achieved by isotope exchange of **5** with sodium iodide-^{125}I in refluxing acetone in a manner similar to that previously employed for the preparation of 19-iodocholesterol-^{125}I.[2a]

Tables I and II compare the concentration of radioactivity at various time intervals for adrenal, blood, kidney, liver, and thyroid following intraperitoneal administration of 19-radioiodinated cholesterol and β-sitosterol to immature male rats (175–200 g). The radioactivity is expressed in dpm/mg of tissue or homogenate and the values are an average of two samples. Values for two rats at each time interval are shown despite the expected variation in tissue counts from one animal to another due to the inability to give exactly the same tracer dose each time as well as to variations in animal eating and excretory habits.

Examination of Tables I and II clearly shows a considerable selective localization of radioactivity in the adrenal gland. While no effort was made to separate adrenal cortex from medulla radioactivity in this preliminary study in rats, experiments with 19-iodocholesterol-^{125}I in dogs have shown that this radioactivity resides almost entirely in the adrenal cortex of the gland.[2b] Thyroid tissue was examined in each instance to provide a measure of the *in vivo* stability of the radioiodinated products. The extremely high radioactivity apparent in the thyroid at early time periods suggests *in vivo* deiodination of the administered compounds. The hazards associated with this characteristic property of most radioiodinated products is overcome clinically by predosing the patient with KI solution.

Because of the unavoidable animal variation in tissue counts mentioned above, it is oftentimes preferable to compare ratios using one tissue as a standard, usually blood or plasma. Since the objective of this study was to find agents suitable for photoscanning the adrenal, it is obviously more advantageous to determine the ratio of radioactivity in the adrenal with that of the nearest interfering organs, liver and kidney. These target to nontarget ratios are shown in Table III. Moreover, Figure 1 shows graphically the average adrenal/liver ratios for 19-iodocholesterol and 19-iodo-β-sitosterol at different time periods. Both radioiodinated

† For paper X, see ref 1.

‡ This study was supported by Grants CA-08349 from the National Cancer Institute, National Institutes of Health, and PRA-18 from the American Cancer Society.

§ Since the product of tritiation was not characterized, it must be assumed that the product was a mixture of tritiated sitosterols and/or sitostanols.

Table I. Distribution of Radioactivity in Tissues of Male Rats at Various Times Following Intraperitoneal Injection of 19-Iodocholesterol-^{125}I

	Time, hr							
	24		48		96		144	
Tissue	Rat 1	Rat 2	Rat 1	Rat 2	Rat 1	Rat 2	Rat 1	Rat 2
Adrenal	14453[a]	15246	4495	44012	25669	3925	8987	53740
Blood	478[b]	536	273	935	431	56	2368	2234
Kidney	372.5	326.5	112	602	248	66	58	145
Liver	1830	1600	329	1533	409	109	63	312
Thyroid	96431	101342	341153	[c]	284577	148477	65197	210325

[a]Values represent average dpm/mg derived from duplicate samples of tissue preparation. [b]Dpm/mg of one sample only. [c]Considerable variation among tissue samples.

Table II. Distribution of Radioactivity in Tissues of Male Rats at Various Times Following Intraperitoneal Injection of 19-Iodo-β-sitosterol-^{125}I

	Time, hr							
	24		48		96		144	
Tissue	Rat 1	Rat 2	Rat 1	Rat 2	Rat 1	Rat 2	Rat 1	Rat 2
Adrenal	9764[a]	12051	31521	2773	26142	47435	333.5	57
Blood	487.5	5725	387.5	68	2716	2343	1558	131
Kidney	231	227	245.5	31	156	193	27	10
Liver	2260	2582	1231.5	153.5	938.5	742.5	9	5
Thyroid	125255	228520	133447	[c]	182360	281516	22535	32360

[a,c]See Table I.

Table III. Target to Nontarget Ratios of Radioactivity Following Administration of 19-Radioiodinated Cholesterol and Sitosterol to Male Rats

	Adrenal/liver		Adrenal/kidney	
Time, hr	Cholesterol	Sitosterol	Cholesterol	Sitosterol
24	7.9, 9.5[a] (8.7)[b]	4.3, 4.7 (4.5)	38.9, 46.8 (42.9)	42.3, 53.1 (47.7)
48	13.7, 28.7 (21.2)	25.6, 17.7 (21.6)	40.1, 73.1 (56.6)	128.4, 87.8 (108.1)
96	62.8, 36 (49.4)	27.9, 63.9 (45.9)	103.5, 59.5 (81.5)	167.6, 245.8 (206.7)
144	142.7, 172.2 (157.4)	37, 11.4 (24.2)	154.9, 370.6 (262.7)	12.4, 5.7 (9.0)

[a]Represents ratios for rats 1 and 2, respectively. [b]Represents average value.

sterols displayed similar adrenal/liver and adrenal/kidney ratio patterns for the first 96 hr. The major difference between the two agents arose at 144 hr. At this time period, the adrenal radioactivity for 19-iodo-β-sitosterol treated rats had begun to dissipate such that the difference between other tissues was declining. In contrast, the adrenal radioactivity for the 144-hr 19-iodocholesterol rats was maintained leading to extremely high target to nontarget ratios. Since retention of radiation for prolonged periods increases the radiation hazard to the adrenals, further studies with 19-radioiodinated β-sitosterol seem warranted.

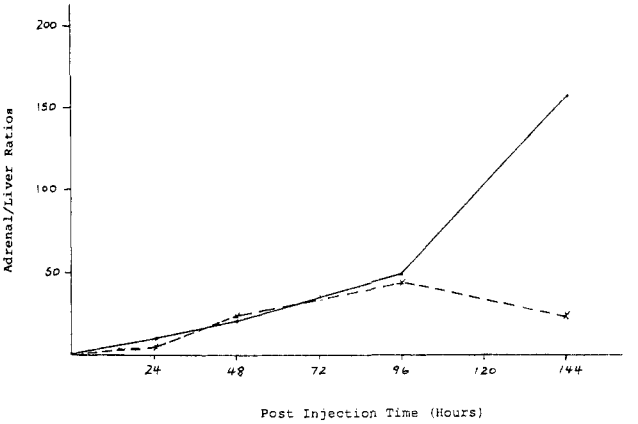


Figure 1. Adrenal liver ratio of radioactivity *vs.* postinjection time (hr) (values represent the average for two animals): (●—●) 19-iodocholesterol-^{125}I; (×—×) 19-iodo-β-sitosterol-^{125}I.

Experimental Section[#]

Sitost-5-ene-3β,19-diol 3-Acetate 19-*p*-Toluenesulfonate (3). A solution of sitost-5-ene-3β,19-diol (**2**, 1.0 g) and *p*-toluenesulfonyl chloride (0.8 g) in dry pyridine (10 ml) was stirred at room temperature overnight and then poured into ice-H_2O. The reaction mixture was extracted with EtOAc and the extract washed with H_2O and dried (Na_2SO_4). Concentration of the EtOAc extract gave a pale yellow solid which was recrystallized from MeOH to give **3** as a colorless solid (0.8 g, 61%): mp 109°; ir and nmr as expected. *Anal.* ($C_{38}H_{58}SO_5$) C, H.

Sitost-5-ene-3β,19-diol 19-*p*-Toluenesulfonate (4). A solution of sitost-5-ene-3β,19-diol 3-acetate 19-*p*-toluenesulfonate (**3**, 1.0 g) in dioxane (35 ml) was added dropwise to a solution of NaOH (500 mg) in aqueous MeOH (50 ml). The solution was stirred at room temperature for 2 hr and then poured into ice-H_2O. The reaction mixture was extracted with Et_2O and the combined Et_2O extract washed with H_2O and dried (Na_2SO_4). Concentration of the Et_2O extract gave a solid which upon recrystallization from Me_2CO-H_2O gave **4** (0.8 g, 81%) as a colorless solid: mp 112–115°; ir and nmr as expected. *Anal.* ($C_{36}H_{56}SO_4$) C, H.

19-Iodositost-5-en-3β-ol (5). A solution of 4 (600 mg) and NaI (300 mg) in *i*-PrOH (40 ml) was gently refluxed for 4 hr under N_2. The solution was concentrated under vacuum to 2 ml and poured into ice-H_2O. The reaction mixture was extracted with Et_2O and

[#]Melting points were taken on a Fisher-Johns melting point apparatus and are corrected. Elemental analyses were performed by Spang Microanalytical Laboratories, Ann Arbor, Mich. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. Ir spectra were taken on a Perkin-Elmer 337 spectrophotometer. The nmr spectra were obtained with a Varian A-60 spectrometer in $CDCl_3$ and TMS as an internal standard. Tlc were run with Eastman chromagrams cut in 1-in. wide strips and spots detected with iodine vapor. Chromatograms of radioiodinated compounds were scanned with an Atomic Associate RCS-363 radiochromatogram scanner. Specific activities were ascertained using a Beckman LS-200 liquid scintillation counter.

the extract washed with H_2O and dried (Na_2SO_4). Concentration of the Et_2O extract left a colorless solid which was recrystallized from Me_2CO to give **5** (400 mg, 72%) as a colorless solid: mp 124°; ir and nmr as expected. *Anal.* ($C_{29}H_{49}IO$) C, H.

19-Iodositost-5-en-3β-ol-^{125}I. A solution of **5** (50 mg) and $Na^{125}I$ (3.8 mCi) in Me_2CO (4 ml) was heated to gentle reflux for 4 hr. The solution was allowed to cool and cold H_2O was added slowly when a solid separated. The solid was collected by filtration and recrystallized from Me_2CO to give 32 mg of 19-iodo-β-sitosterol-^{125}I with a specific activity of 66.0 μCi/mg (55% exchange). Tlc using $CHCl_3$–EtOH (1:1) (R_f 0.64) or C_6H_6–EtOAc (1:1) (R_f 0.43) showed a single spot coincident with a single radioactive peak appearing on the radiochromatogram scan.

Tissue Distribution Studies. Radioiodinated steroids were given by intraperitoneal injection to immature male Sprague-Dawley albino rats weighing 175–200 g. The dose administered was approximately 50 μCi per rat and the vehicle used was 90% EtOH (0.2–0.3 ml). Groups of two animals were killed by exsanguination through the ventricle 24, 48, 96, and 144 hr after the injection. The major organs such as liver and kidney were excised, weighed, and homogenized. These organs were washed thoroughly with isotonic saline to remove blood, dried, and minced with scissors. Minced tissue was then placed in a homogenizer tube containing 20 ml of H_2O in the case of liver and 3 ml of H_2O in the case of kidney. Homogenates were not prepared for small organs such as adrenal and thyroid. Liver and kidney homogenates and entire adrenal, thyroid, and several heparinized blood samples were placed in scintillation counting vials. To each vial 0.5 ml of 10% NaOH solution was added and left overnight and then heated for at least 10 min at 60° in a water bath to complete digestion. The vials were allowed to cool and 5 drops each of glacial HOAc and 30% H_2O_2 solution were added. Ten milliliters of thixotropic liquid-counting system[10] was then added to each vial and the contents were shaken using a vortex mixer. The vials were kept in a cool dark place for at least 4 hr before counting. Radioactivity was assayed in a Beckman LS-200 liquid scintillation spectrometer. Sufficient counts were accumulated to reduce the probable error of counting to less than 5%. All counts were corrected for quench by using ^{125}I-quench standards curves.

References

(1) G. N. Holcomb, C. M. Boyd, R. E. Counsell, W. H. Beierwaltes, R. A. Szczesniak, D. R. Murty, and G. A. Bruno, *J. Pharm. Sci.*, **60**, 390 (1971).
(2) (a) R. E. Counsell, V. V. Ranade, R. J. Blair, W. H. Beierwaltes, and P. A. Weinhold, *Steroids*, **16**, 318 (1970); (b) R. J. Blair, W. H. Beierwaltes, L. M. Lieberman, C. N. Boyd, R. E. Counsell, P. A. Weinhold, and V. M. Varma, *J. Nucl. Med.*, **12**, 176 (1971).
(3) L. M. Lieberman, W. H. Beierwaltes, J. W. Conn, A. N. Ansari, and H. Nishiyama, *N. Engl. J. Med.*, **285**, 1387 (1971).
(4) J. W. Conn, R. Morita, E. L. Cohen, W. H. Beierwaltes, W. J. McDonald, and K. Herwig, *Arch. Int. Med.*, **129**, 417 (1972).
(5) W. H. Beierwaltes, L. M. Lieberman, A. N. Ansari, and H. Nishiyama, *J. Amer. Med. Ass.*, **216**, 275 (1971).
(6) R. Morita, L. M. Lieberman, W. H. Beierwaltes, J. W. Conn, A. N. Ansari, and H. Nishiyama, *J. Clin. Endocrinol. Metab.*, **34**, 36 (1972).
(7) T. Nagai, B. A. Solio, and C. S. Koh, *J. Nucl. Med.*, **9**, 576 (1968).
(8) E. Denot, C. Casas-Campilio, and P. Crabbe, *Eur. J. Steroids*, **2**, 495 (1967).
(9) A. Bowers, R. Villotti, J. A. Edwards, E. Denot, and D. Halpern, *J. Amer. Chem. Soc.*, **84**, 3204 (1962).
(10) E. P. Frenkel, B. E. Whalley, C. T. Knorp, and D. R. Korst, *J. Lab. Clin. Med.*, **59**, 174 (1962).

Substituted Tetralines. 5. Analgesic Properties of Some Diastereoisomeric *N,N*-Dimethyl-4-phenyl-1,2,3,4-tetrahydro-2-naphthylamines[1]

E. M. Kandeel and A. R. Martin*

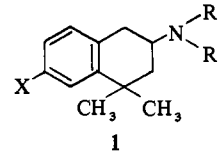
College of Pharmacy, Washington State University, Pullman, Washington 99163.

Received March 1, 1973

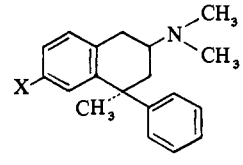
In a series of recent publications the synthesis and analgesic potency of some derivatives of general structure **1** were reported.[2-4] As part of a continuing study of structure-activity relationships directed toward the investigation of the effect of 4 substitutions in this system, the diastereoisomeric *N,N*-dimethyl-4-phenyl-1,2,3,4-tetrahydro-2-naphthylamines **2a** and **2b** and their 6-methoxyl and 6-hydroxyl derivatives **3a,b** and **4a,b**, respectively, were prepared and tested for analgesic activity.

Substitution of a phenyl group for methyl in **1** provides analogs of the diaryl analgesics, *e.g.*, methadone (**5**) and thiambutene (**6**), wherein the conformation of one of the phenyl rings is partially restricted. If it is assumed that the tetralin ring system (present in virtually all potent analgesics having rigid structures[5]) serves in the role as a determinant of analgesic potency proposed by Beckett and Casy,[6] then 4-phenyl-substituted tetralins might be useful for investigating the importance of the second phenyl group of diaryl analgesics.

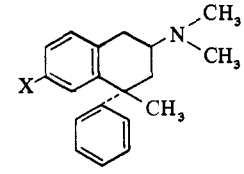
Chemistry. The amines **2a**, **2b**, **3a**, and **3b** were prepared from the corresponding 4-methyl-4-phenyl-1,2,3,4-tetrahydro-2-naphthoic acids **7a**, **7b**, **8a**, and **8b** by means of a modified Curtius procedure,[7] followed by Eshweiler-Clarke methylation[8] of the primary amines. The synthesis, separation, and proof of stereostructure of the diastereoisomeric acids, **7** and **8**, have been reported previously.[9] Treatment of the *N,N*-dimethyl-6-methoxy-4-methyl-4-phenyl-1,2,3,4-tetrahydro-2-naphthylamines **3a** and **3b** with 48% HBr gave **4a** and **4b**, respectively.



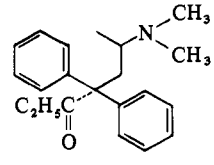
1



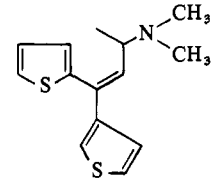
2a, X = H
3a, X = OCH_3
4a, X = OH



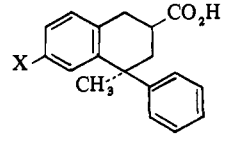
2b, X = H
3b, X = OCH_3
4b, X = OH



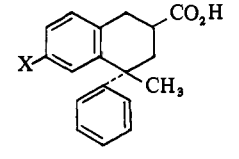
5



6



7a, X = H
8a, X = OCH_3



7, X = H
8, X = OCH_3

Biological Results. The analgesic potencies of the amines were determined in white albino mice (Carnworth Farm CF No. 1 strain) by the hot-plate procedure of Eddy and Leimbach.[10] In the complete assay procedure for each compound, five groups of six mice each were screened at dosage levels of 0.025, 0.050, 0.075, and 0.100 mmol/kg and a normal