pounds were nontoxic to mice. Apparently there was no correlation between the water solubility and antimalarial activity of these derivatives.

Experimental Section

Melting points were taken on a Thomas-Hoover Unimelt apparatus and are corrected. A Beckman IR-8 spectrophotometer was used to determine the ir spectra. The uv spectra were obtained on a Cary-14 spectrophotometer. The nmr spectra were run on a Varian A-60 spectrometer using Me_4Si as internal standard. The elemental analyses were performed by Midwest Microlab, Inc., Indianapolis, Ind. Where analyses are indicated only by symbols of the elements, analytical results were within ±0.4% of the theoretical values.

Preparation of the Sulfate Ester 1b. A suspension of SO_3–pyridine complex was prepared by stirring dry pyridine (30 ml) and $ClSO_3H$ (3.5 g, 0.03 mol) in a 300-ml round-bottom flask cooled in an ice bath. A solution of 1a·HCl obtained by warming 5.4 g (0.01 mol) of this compound in dry pyridine (70 ml) was added to the above suspension dropwise with stirring. The resulting mixture was refluxed for 30 min with stirring and kept at room temperature overnight. The pyridine was removed by evaporation under reduced pressure and the residual white cake extracted several times with $CHCl_3$ (300 ml). The $CHCl_3$ extract was washed with H_2O (5 × 100 ml), dried (Na_2SO_4), and evaporated under reduced pressure to dryness. The resulting white solid was crystallized from $CHCl_3$ to give 5.1 g (86%) of 1b, mp 225–227° dec. *Anal.* ($C_{30}H_{42}BrNO_4S$) C, H, N, S. The ir spectrum ($CHCl_3$) showed strong absorption around 1140–1320 cm^{-1} (OSO_3H) and no absorption in the OH region. The uv spectra of compound 1a·HCl and 1b were very similar: 1a·HCl (1.27×10^{-5} mol, $CHCl_3$) λ_{max} 343 nm (ϵ 2600), 327 (3070), 294 (35,400), 282 (30,700); 1b (1.33×10^{-5} mol, $CHCl_3$) λ_{max} 343 nm (ϵ 2630), 327 (3080), 294 (35,380), 282 (30,800).

Preparation of Di(*O*-phenylphosphoro)-6-bromo-α-(di-*n*-heptylaminomethyl)-9-phenanthrenemethanol (1c). Diphenyl phosphorochloridate (3.2 g, 0.01 mol) was added to a solution of 1a·HCl (4.9 g, 0.009 mol) in dry pyridine (75 ml) at 0°. After keeping the solution at 0° for 18 hr the pyridine was removed by evaporation under reduced pressure. The residue was dissolved in $CHCl_3$ (200 ml); the $CHCl_3$ layer was washed with H_2O (5 × 100 ml), dried (Na_2SO_4), and evaporated to dryness under reduced pressure. Upon crystallization from $CHCl_3$–pentane, the residue yielded 6.06 g (91%) of compound 1c, mp 100–103°. *Anal.* ($C_{42}H_{51}BrNO_4P$) C, H, N, P. Its ir spectrum had no absorption around 3320 cm^{-1} (OH) and strong absorptions at 1208, 1228, 1090, and 910 cm^{-1} ($OPOC_6H_5$); nmr ($CDCl_3$) δ 7.2–8.8 (m, 18, Ar H), 3.1 [m, 6, $-CH_2N(CH_2)_2$], 1.2 [m, 21, $C(CH_2)_5C)_2$, OCH–], 0.9 [(m 6, $(CCH_3)_2$].

Preparation of Di(*p*-nitrophenyl) Phosphorochloridate. Diphenyl phosphorochloridate (16.5 g, 0.06 mol) was dissolved in dry CCl_4 (50 ml) in a three-necked round-bottom flask equipped with a stirrer, thermometer, dropping funnel, and drying tube. A mixture (14.6 ml) of 30% HNO_3 and 70% H_2SO_4 was added to it dropwise so as to keep the reaction mixture at 5–15°. After stirring at 5–15° for 4 hr the reaction mixture was extracted with dry CH_2Cl_2 (300 ml). The CH_2Cl_2 solution was neutralized with anhydrous $CaCO_3$. The solid was removed by filtration and the solvent removed *in vacuo*. The residue was dissolved in a small amount of $CHCl_3$, followed by addition of petroleum ether (bp 30–60°) into the flask until an oily layer separated out. On keeping the flask in the refrigerator overnight, the title compound crystallized out as white prisms: yield 12.1 g (62%); mp 90–92° (lit.[8] mp 97–97.5°).

Preparation of Di(*O*-*p*-nitrophenylphosphoro)-6-bromo-α-(di-*n*-heptylaminomethyl)-9-phenanthrenemethanol (1e). To a solution of compound 1a·HCl (1.1 g, 0.002 mol) in dry pyridine (30 ml) was added a solution of di(*p*-nitrophenyl) phosphorochloridate (0.75 g, 0.002 mol) in dry pyridine (10 ml). The resulting mixture was kept at room temperature overnight. The pyridine was removed *in vacuo* and the resulting yellow solid dissolved in $CHCl_3$ (100 ml) and washed with H_2O (3 × 30 ml). The $CHCl_3$ solution was dried (Na_2CO_3) and evaporated under reduced pressure. The residue on crystallization from $CHCl_3$–pentane gave compound 1e as pale yellow needles: 1.2 g (72%); mp 87–90°; ir (Nujol) 1083 (s), 1103 (w), 1170 (m), 1229 (s), 1260 cm^{-1} (s, *O*-*p*-nitrophenyl), 1349 (s, CNO_2), 1520 and 1600 (s, CN=O); nmr ($CDCl_3$) δ 0.8–3.4 (m, 33, aliphatic), 7.2–8.8 (m, 16, Ar H). *Anal.* ($C_{42}H_{39}BrN_3O_8P$) H, N, P; C: calcd, 60.43; found, 58.91.

Alkaline Hydrolysis of 1e. A solution of 1e in 95% EtOH (10 ml) was treated with alcoholic KOH (1 g dissolved in 15 ml of 95% EtOH) at room temperature for 2 hr. Work-up afforded the isolation of only potassium di(*p*-nitrophenyl) phosphate, which was identified by comparison with an authentic sample (identical ir spectrum).

General Procedure for the Preparation of the Dicarboxylic Acid Salts of Compound 1a. A solution of 1a·HCl (11.0 g, 0.03 mol) in 250 ml of CH_2Cl_2 was vigorously stirred with an aqueous NaOH solution (1 *N*, 100 ml) for 30 min. The CH_2Cl_2 layer was separated and washed with 50 ml of H_2O, dried (Na_2CO_3), and evaporated under reduced pressure at room temperature. The residue containing the free base 1a was then treated with a solution of the dicarboxylic acid (0.02 mol) in 70 ml of absolute EtOH and 1 ml of H_2O. The reaction mixture was heated over steam bath for 5 min, cooled, and kept at room temperature for 4 hr. After removal of EtOH *in vacuo*, absolute ether (50 ml) was added and the flask kept in the refrigerator overnight. White crystals of the salt which separated out were collected by filtration. The salts were recrystallized from absolute EtOH–ether. The yields, ir spectra, and analyses of the salts are listed in Table I.

Preparation of 1f. Glutaric anhydride (4.0 g, 0.0035 mol) was added to a solution of 1a HCl (16.5 g, 0.03 mol) in dry pyridine (250 ml). The mixture was refluxed for 12 hr. The pyridine was removed *in vacuo* and the resulting yellow residue dissolved in 250 ml of $CHCl_3$ and washed with H_2O (5 × 100 ml). The $CHCl_3$ solution was dried (Na_2CO_3) and evaporated to dryness under reduced pressure The residue on crystallization from ether–pentane gave compound 1f as a white solid (15.0 g, 80%), mp 79–81°. *Anal.* ($C_{35}H_{48}BrNO_4$) C, H, N.

General Procedure for the Determination of Distribution Coefficients of the Dicarboxylic Acid Salts and Esters of Compound 1a. Stock solutions were prepared by dissolving 10 mg of each sample in 250 ml of 1-octanol at room temperature. The optical density of the solution at 291 nm was measured using a Cary-14 uv spectrophotometer. In each case 10 ml of the stock solution was shaken with 500 ml of distilled water for 3 min. After letting it stand for 30 min at room temperature to ensure that the equilibrium had been reached, the organic layer was separated, dried (Na_2SO_4), and filtered, and the optical density of the solution was measured again. From the differences in the optical density of each solution before and after shaking with H_2O, the equilibrium concentrations of the compound in 1-octanol and water were calculated. The distribution coefficients given in Table II were calculated for these concentrations. Recently, Higuchi and Pitman[9] had also reported the use of absorbance values for calculating the equilibrium concentrations of dissolved caffeine.

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Mechanism of Narcosis. Entropy of Hydration of Gaseous General Anesthetics

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One of the most widely studied, yet poorly understood phenomena in the realm of neurochemistry may well be that of general anesthesia. The chemical agents responsible for this type of narcosis span a wide spectrum of chemical

Table I. Anesthetic Pressures and Some Physical Properties of Gases

Gas	Anesthetic pressure, atm[a]	Concn in olive oil at anesthetic pressure, mol/l.[b]	Henry's constant, for gas in H_2O, 25° (atm), × 10^{-4}[c]	$N^d \times 10^4$	ΔS_H,[e] cal/deg[f]	$\frac{-\Delta S - 6.30}{\log 1/N}$
Ar	20	0.12	4.10	4.88	−31.1	7.49
Kr	2.9	0.055	2.35	1.23	−32.9	6.80
Xe	0.85	0.063	1.30	0.654	−35.8	7.05
N_2	29	0.084	8.63	3.36	−32.1	7.43
CH_4	4.6	0.10	3.92	1.17	−33.3	6.87
C_2H_4	0.80	0.044	1.14	0.70	−32.6	6.33
C_2H_2	0.70	0.054	0.134	5.22	−26.8	6.25
SF_6	3.0	0.032	22.8	0.132	−41	7.11
N_2O	0.9	0.056	0.228	3.95	−31.6	7.43
Ne	88		12.4	7.10	−29.2	7.28
C_2H_6	1.3		2.90	0.448	−38.6	7.43
C_3H_8	0.9		3.83	0.234	−40.0	7.28
CF_4	15		27.6	0.543	−37	7.20
CH_3Cl	0.14		0.0524	2.67	−31.5	7.05
CH_3I	0.07		0.0296	2.36	−32.6	7.25

[a] From L. J. Mullins, *Chem. Rev.*, **54**, 289 (1954); W. F. Van Oetinger, "The Halogenated Hydrocarbons, Toxicity and Potential Dangers, Public Health Service Publication 414," U. S. Government Printing Office, Washington D. C., 1955; and from ref 3 and 8. [b] From ref 13. [c] From T. J. Morrison and N. B. Johnstone, *J. Chem. Soc.*, 3441 (1954); E. Douglas, *J. Phys. Chem.*, **68**, 169 (1964); W. F. Clausen and M. F. Polglase, *J. Amer. Chem. Soc.*, **74**, 4817 (1952); and "Handbook of Chemistry," 9th ed, N. A. Lange, Ed., Handbook Publishers, Sandusky, Ohio, 1956. [d] N = mole fraction of gas dissolved in H_2O at anesthetic pressure. [e] Excess molar entropy of the gas in an aqueous solution at concentration, $N = 1$, over the molar entropy of the gas at 1 atm and 25°. [f] From J. T. Ashton, R. A. Dawe, K. W. Miller, E. B. Smith, and B. J. Stickings, *J. Chem. Soc.*, 1793 (1968); D. W. Glew and E. A. Moelwyn-Hughes, *Discuss. Faraday Soc.*, **15**, 150 (1953); R. A. Pierotti, *J. Phys. Chem.*, **69**, 281 (1965); J. A. V. Butler, *Trans. Faraday Soc.*, **33**, 229 (1937).

structures and physical and chemical properties. They generally lack the complex functional and stereochemical specificities characteristic of biochemically active substances. They include among their number the simplest substances of all, *i.e.*, the rare gases. This study concerns itself with a number of volatile anesthetics of the rare gas type, namely, the rare gases themselves and a number of other biochemically inert gases.

The oldest and also the most widely quoted hypothesis concerning narcotic activity is that of Meyer[1] and Overton.[2] This hypothesis is based on the observation that there is a rough proportionality between the lipid solubility and the anesthetic potency of many general anesthetics. No specific mechanism of action is proposed in this theory, but the site of action of the narcotic agent is considered to be in the lipid portion of the neuronal membrane. The larger the concentration of the agent in the lipid phase, the greater should be its effectiveness. Thus, a particular concentration of one narcotic agent should have the same effectiveness in producing narcosis as any other agent regardless of any differences between them in chemical or physical properties (other than the lipid-water partition coefficient, which determines the concentration in the lipid phase). This hypothesis is the one most frequently mentioned in standard treatments of the subject and is also supported by recent investigators.[3-5] In attempting to evaluate the degree to which this hypothesis fits the facts for the case of gaseous anesthetics, one must therefore calculate the concentration of the narcotic gas in the lipid phase at the pressure required for general anesthesia. A plot of P_A *vs.* P_A/C_L (where P_A is the anesthetic pressure of the gas and C_L is the concentration of the gas in the lipid phase) should then yield a straight line whose slope is equal to the effective anesthetic concentration of all gases in the lipid phase. The first three columns of Table I contain a typical example of data of this kind. As can be seen, the concentration of the narcotic agent in the lipid is far from constant (Figure 1). The standard deviation, $\sigma = 0.027$, which is 39.7% of the mean value. The use of other literature values for anesthetic pressure[3,6] does not yield a better correlation.

Ten years ago Pauling[7] and, independently, Miller[8] proposed a theory which places the site of action of the narcotic agent in the aqueous phase of the central nervous system. These two proposals have the advantage of containing more definite speculations concerning a mode of action of general anesthetics than had been made hitherto. Starting with the observation that certain inert gases, which are also anesthetics, form hydrates, both authors suggested that the function of the anesthetic agent was to increase the order of the aqueous phase of the neuron, thus lowering its conductance.

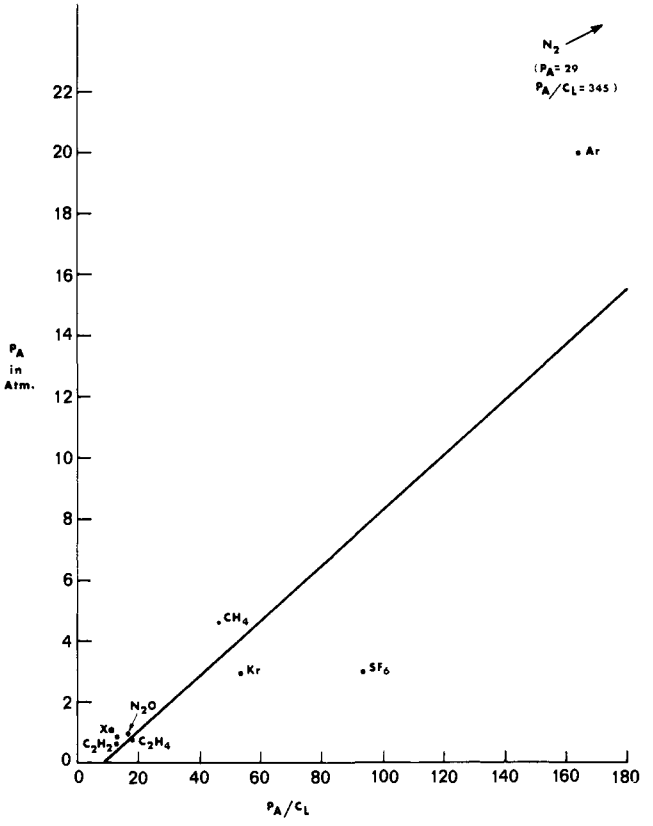

Figure 1. Plot of P_A *vs.* P_A/C_L (where P_A is the anesthetic pressure of the gas and C_L is the concentration of the gas in the lipid phase).

A direct measure of the degree of order introduced into an aqueous phase by a dissolved gas should be the entropy of solution of the gas in water. Table I lists values for the entropy of solution in water, ΔS_H, of 15 anesthetic gases. To evaluate the degree to which this correlates with narcotic activity one must compare these ΔS_H values with the amount of gas dissolved in the aqueous phase at the anesthetic pressure of each gas. Using the Henry's law constant, K_H, the mole fraction ($N = P_A/K_H$) of each gas in the aqueous phase at the anesthetic pressure was calculated (Table I). If the degree of order in the aqueous phase is the governing factor, then there should be an inverse relationship between this mole fraction [N] and a function describing the degree of order. Since $-\Delta S_H$ is an exponential function of the degree of order [$S = k \ln \Omega$], the correlation should be between the log of $1/N$ and $-\Delta S_H$.

Using the first nine gases only (the same nine for which data testing the Meyer-Overton theory are available), we found a linear correlation between $-\Delta S_H$ and log $1/N$. A least-squares plot yields the equation

$$-\Delta S = 6.54 \log 1/N + 7.93 \quad (1)$$

Using all 15 gases for which data are available, we obtain the equation

$$-\Delta S = 7.08 \log 1/N + 6.30 \quad (2)$$

The slopes for the individual points calculated from the intercept of this equation are listed in the last column of Table I. The standard deviation for the first nine points is σ = 0.44 which is 6.2% of the mean value. This is clearly a vast improvement over the correlation based on the Meyer-Overton hypothesis.† The standard deviation for all 15 points is σ = 0.37 or 5.2% of the mean value (Figure 2). This is a remarkably close correlation, particularly in view of the difficulty in obtaining good experimental values of P_A and ΔS_H. The broad range of anesthetic pressures covered by this correlation is also worth noting.

The entropy correlation reported above is also quantitatively superior to the correlation of anesthetic potency with the gas hydrate dissociation pressure originally reported by Miller[8] and by Pauling.[7] The above authors did not suggest that gas hydrate crystals are actually formed under physiological conditions[8] but rather that in the presence of the dissolved anesthetic gas the aqueous phase formed regions of order which they termed "icebergs"[8] or "microcrystals."[7] The gas hydrate dissociation pressure was seen as a measure of this ordering capacity of the dissolved anesthetic gas. We concur with their views and suggest that the entropy of hydration is a better measure of the ordering of the aqueous phase. We believe that the entropy correlation therefore supports the following conclusions: (1) that the site of action of gaseous general anesthetics is to be found in an aqueous portion of the central nervous system, most probably in the axoplasm; and (2) that the mode of action may well involve the formation in the axoplasm of more highly ordered structures of the kind suggested by Pauling and by Miller.

Finally, one might ask whether the correlation reported here involves a physical effect of an order of magnitude such as might reasonably be expected in terms of current theories of nerve impulse conduction. The intercept in eq 2, 6.30 eu, corresponds to the ΔS_H of a hypothetical narcotic agent which would be effective only if it completely displaced the aqueous phase, *i.e.*, where log $(1/N) = 0$, or $N = 1.0$. The value of −6.30 eu/mol of aqueous phase is therefore a measure of the amount of order necessary to induce narcosis. A change of 6.30 eu/mol corresponds to a free energy change of 1877 cal/mol [6.30 cal/(deg mol) × 298°] which in turn corresponds to a change in potential of 0.081 V (1877 cal/mol × 4.184 J/cal ÷ 96,500 C/mol). It is most suggestive that this potential change is of the same order of magnitude as the resting potential of the neuronal membrane, 0.05–0.07 V.[10] Does the presence of the narcotic agent diminish the resting potential, thus inhibiting the conduction of the nervous impulse by a process of depolarization analogous to that observed during the refractory period immediately following the passage of an impulse? The association of neuromuscular block with depolarization of the membrane is well established. The effect of narcotizing solutions in raising the threshold value of isolated nerves has been observed as early as 1939 by Tasaki.[11] The order of magnitude of the effect observed by him and also by later workers[12,13] agrees well with our calculated value. The objection may be raised that gaseous anesthetics are believed to interfere mainly with transmission at the synapse and that, therefore, they function by interfering with the chemical transmitters at the synapse rather than by interfering with electrical conduction. It has, however, been shown by Carpenter[13] that the blocking mechanism at the synapse is the same as that in isolated fibers, *i.e.*, that a reversible decrease in the polarization potential followed exposure to the anesthetic gas. Whatever the detailed mechanism may be, the quantitative correspondence between the magnitude of our entropic effect and the magnitude of the depolarization in neuromuscular block is most striking.

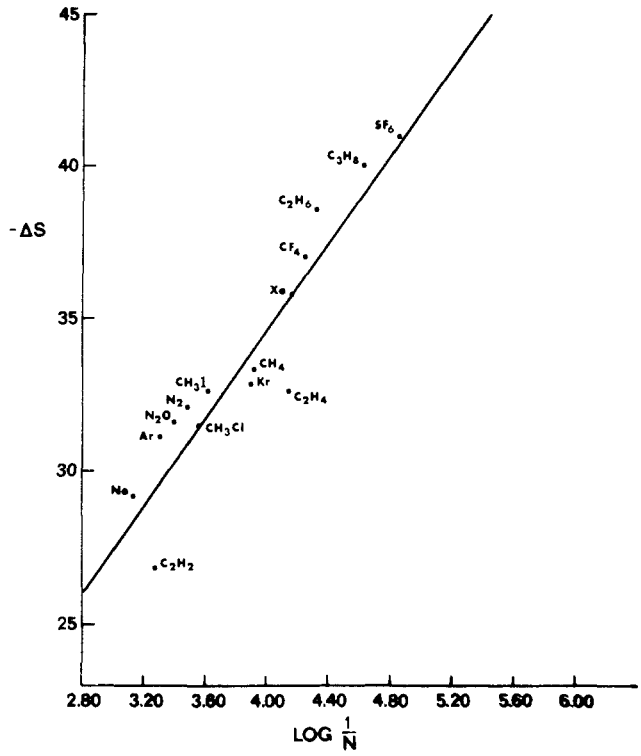

Figure 2. Plot of log $1/N$ *vs.* $-\Delta S$ (where N = mole fraction of gas dissolved in water at the anesthetic pressure and ΔS = the entropy of solution of the gas in water).

†A somewhat better Meyer–Overton correlation (σ = 0.38, which is 18% of the mean, for the product of anesthetic pressure and oil/gas partition coefficient) was obtained by Eger, Lundgren, Miller, and Stevens.[9] Unfortunately, we lack N values for 7 of the 11 anesthetic agents used in his study and therefore this correlation cannot be compared with our entropy correlation.

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

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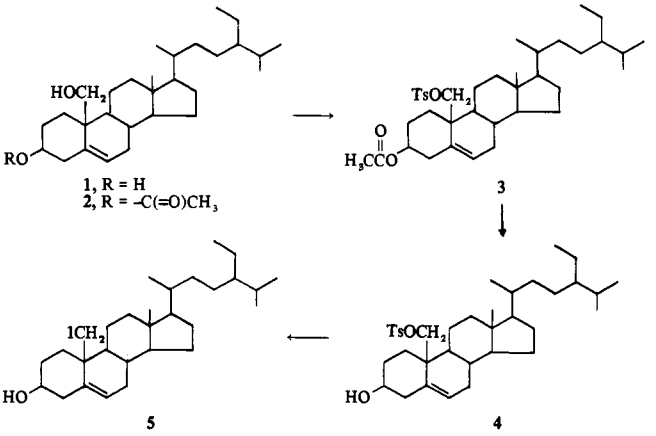
Over the past several years an effort has been made in our laboratory to develop a radiopharmaceutical suitable for photoscanning the adrenal gland and associated tumors. Recently, we reported that 19-iodocholesterol-^{125}I selectively concentrated in the adrenal glands of dogs in a manner similar to 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.

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