Potential Organ- or Tumor-Imaging Agents XX: Ovarian Imaging with 19-Radioiodinated Cholesterol

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Abstract □ Cholesterol is stored in the ovaries where it is a precursor in the biosynthesis of the female sex hormones progesterone and estradiol. The purpose of this study was to determine whether a radioiodinated analog of cholesterol, ^{125}I-labeled 19-iodocholesterol, would mimic cholesterol in this capacity and thus offer a means for imaging the ovaries of intact animals. Aside from the thyroid, the adrenal glands and ovaries of the female rat retained the highest levels of radioactivity at 4 days following the intravenous administration of ^{125}I-labeled 19-iodocholesterol. Moreover, the levels of radioactivity were sufficient to obtain clear images of the adrenal glands and ovaries with a small animal scanner. Folch analysis of these tissues revealed that >80% of the radioactivity was associated with the sterol ester fraction.

Keyphrases □ Cholesterol—19-radioiodinated analog, evaluation as ovarian imaging agent □ Radionuclide imaging—19-radioiodinated cholesterol, evaluation as ovarian imaging agent

19-Radioiodinated cholesterol originally was synthesized as an adrenal imaging agent (1). Since that time, this agent and its close relatives have been used in clinical diagnostic procedures for visualization of the adrenal glands and associated tumors (2).

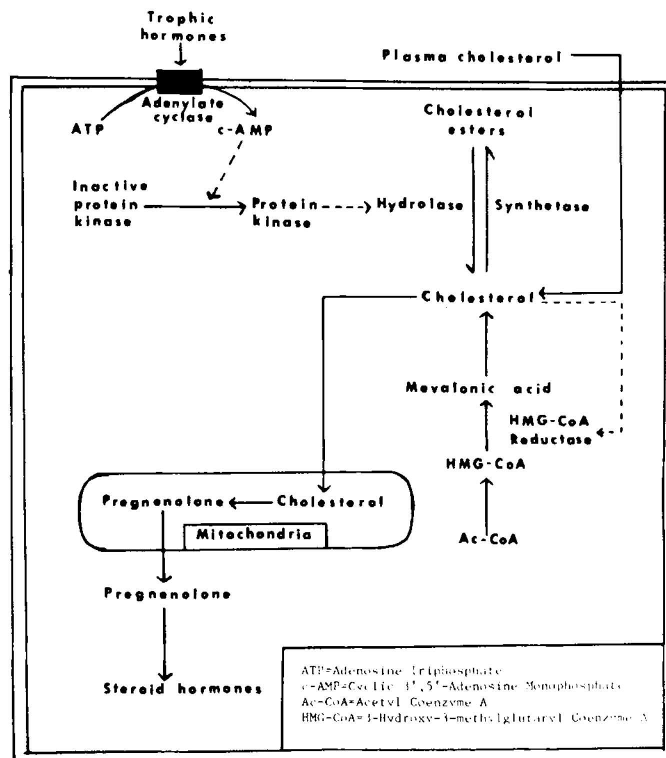
The synthesis of radioiodinated cholesterol as a radiodiagnostic agent was based on the knowledge that the adrenal cortex stores cholesterol for ultimate use in corticosteroid hormone biosynthesis. The ovary is another mammalian organ that is rich in cholesterol and that utilizes this lipid as a precursor for steroid hormone synthesis.

It is generally accepted that the synthesis of steroid hormones in the adrenal gland and ovary is controlled mainly by the trophic hormones adrenocorticotropic hormone and luteinizing hormone, respectively. While many details regarding their modes of action are unclear, they appear to influence the conversion of stored cholesterol ester to free cholesterol and the transformation of the latter compound to pregnenolone (Scheme I) (3).

This similarity between the ovary and the adrenal gland in the storage and utilization of cholesterol suggested that 19-iodocholesterol might accumulate in the ovaries and steroid-secreting ovarian tumors in addition to its predilection for adrenal cortical tissue. Thus, the purpose of this study was to compare the disposition of 19-radioiodinated cholesterol in the adrenal and ovarian tissues of the female rat.

EXPERIMENTAL

Radioiodinated Cholesterol—^{125}I-Labeled 19-iodocholesterol was prepared as described previously (1), yielding 14 mg with an estimated specific activity of 400 μCi/mg. The compound was dissolved in 5.0 ml of benzene and stored at 4° until just prior to use, at which time the material was formulated for injection as follows. An aliquot of the benzene solution was placed in a multidose vial, and the solvent was evaporated. The compound was redissolved in saline containing 1.6% polysorbate 80 and 10% ethanol. Prior to use, one aliquot of the solution was assayed for radioactivity to determine the radioactive concentration, and another



Scheme I

aliquot was analyzed by TLC using silica gel G plates[1] developed in benzene–ethyl acetate (9:1).

Two solutions were prepared. Solution A, used for tissue distribution studies, was made using 1 ml of the benzene solution. The final volume of this solution was 10 ml (103 μCi/ml). Solution B, used for scanning, was prepared using 2.0 ml of the benzene solution. The final volume of this solution was 5.0 ml (350 μCi/ml). Both solutions showed only one radioactive peak when scanned with a radiochromatogram scanner[2]. The location of this peak was coincident with that of the authentic unlabeled material cochromatographed with the radioiodinated sample.

Tissue Distribution—Ten adult female Sprague–Dawley rats[3], 173–185 g, each received an intravenous injection of 25.8 μCi of ^{125}I-labeled 19-iodocholesterol in 0.25 ml of vehicle (Solution A) *via* the tail vein while under ether anesthesia. Rats were either in estrus or metestrus at the time of injection as determined by vaginal smear. Half of the rats were killed 1 day postadministration and half were killed 4 days postadministration by exsanguination from the heart while under ether anesthesia.

Tissues of the adrenal cortex and medulla, liver, ovary, blood, uterus, and thyroid were removed at sacrifice. Samples of the adrenal cortex, ovary, liver, and blood from each rat were placed on ice for subsequent extraction studies. All tissue samples were placed in tared cellulose acetate capsules and weighed. The capsules then were placed in polystyrene γ-counting tubes and assayed for radioactivity in a well scintillation counter[4] for 10 min or until 100,000 counts had accumulated. Counting efficiency for iodine 125 was 87% in all cases, except when glass culture

[1] Eastman Kodak, Rochester, N.Y.
[2] Berthold model 6000.
[3] Spartan Research Animals, Hasslett, Mich.
[4] Searle 1185 γ-spectrometer.

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Table I—Distribution of Radioactivity following Intravenous Administration of ^{125}I-Labeled 19-Iodocholesterol to Female Rats

Tissue	Percent of Dose per Gram[a] 1 Day Postinjection	4 Days Postinjection
Adrenal cortex	10.195 ± 0.251	9.572 ± 0.349
Adrenal medulla	3.002 ± 0.438	1.990 ± 0.554
Blood	0.252 ± 0.010	0.021 ± 0.002
Liver	0.431 ± 0.026	0.059 ± 0.003
Ovary	5.929 ± 0.343	2.736 ± 0.437
Uterus	0.238 ± 0.010	0.055 ± 0.005
Thyroid	199.096 ± 18.263	152.042 ± 10.606

[a] Values represent the mean ± *SEM* for five rats.

tubes were used to hold samples. In the latter case, the counting efficiency was 77%.

Folch Extraction—Radioactivity was extracted from selected tissues using a variation of the procedure described by Folch *et al.* (4). The samples of the liver, adrenal cortex, and ovary were weighed and placed in a ground-glass homogenizer with sufficient distilled water to bring the combined volume of tissue and water to 0.5 ml (1 g of tissue was assumed to be equal to 1 ml). The samples were homogenized in 2–5 ml of chloroform–methanol (2:1) and filtered through filter paper[5]. The homogenizers then were rinsed twice with 2–4 ml of the solvent, and these rinses were filtered. The combined volume of the homogenizing vehicle plus the rinses for each sample was 10 ml.

Aliquots of blood (0.5 ml) were shaken with 5 ml of chloroform–methanol (2:1) in a conical centrifuge tube. The solvent was filtered, and the tube was rinsed with an additional 5 ml of solvent, which also was filtered and combined with the original filtrate. Similar to the tissue samples, the volume of the combined filtrates for each blood extract was measured and adjusted to 10 ml by addition of chloroform–methanol (2:1).

The filter paper with each precipitate was air dried, placed in a polystyrene γ-tube, and assayed as described to ascertain radioactivity associated with precipitable material. Each filtrate was placed in a 15-ml conical centrifuge tube. Distilled water (2.0 ml) was added, and the tube was shaken. The tubes then were centrifuged at low speed for 10 min to separate the phases. The upper phase of each sample was removed; the lower phase was washed with two 1.0-ml portions of Folch's upper phase solvent (chloroform–methanol–water, 3:48:47), which were combined with the upper phase from each sample. Each upper phase was adjusted to 10 ml by addition of the upper phase solvent, and a 1.0-ml aliquot of each upper phase was placed in a polyethylene microfuge tube, which then was placed in a polystyrene γ-tube and assayed for radioactivity as described.

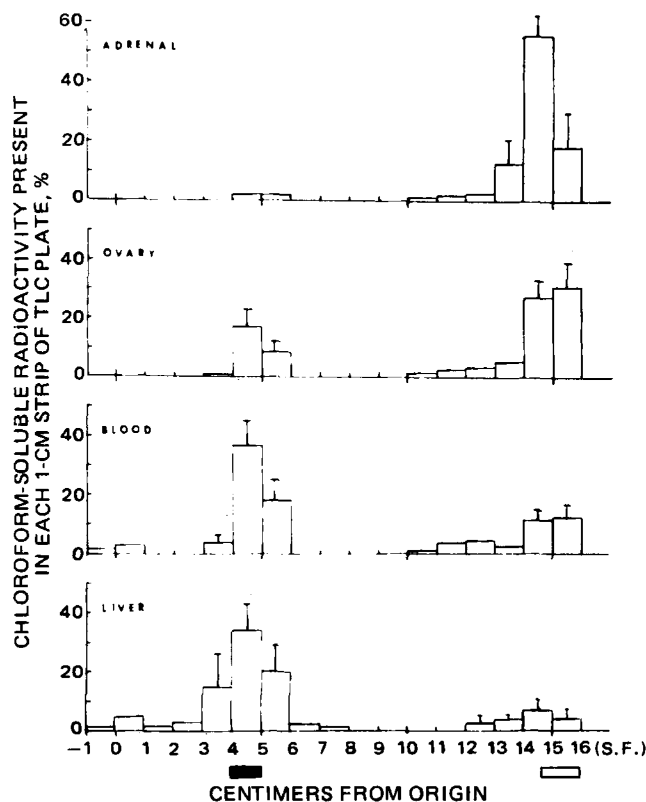

Figure 1—*TLC analysis of lower chloroform phase radioactivity at 1-day interval. Key: ■, free sterol standards; and □, esterified standards. S.F. = solvent front.*

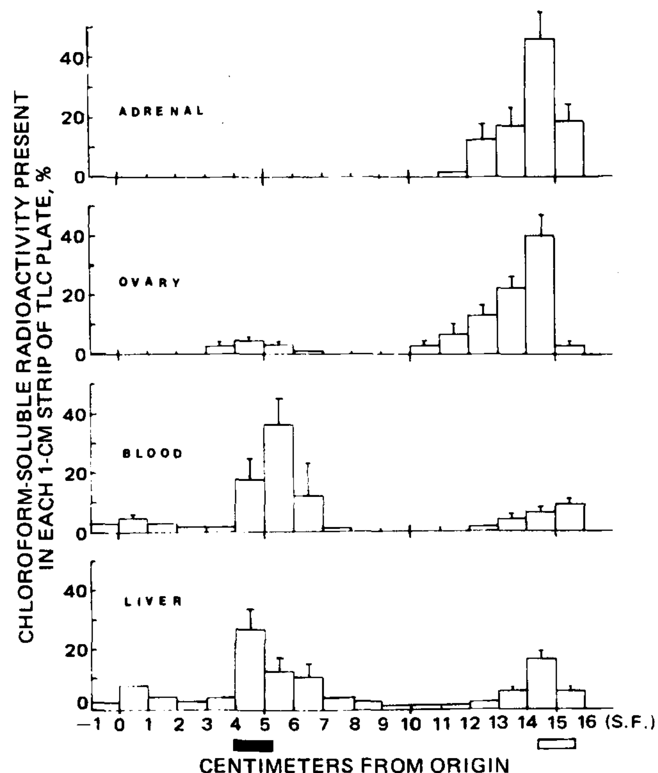

Figure 2—*TLC analysis of lower chloroform phase radioactivity at 4-day interval. Key: ■, free sterol standards; and □, esterified standards. S.F. = solvent front.*

Each lower phase was evaporated to dryness under nitrogen at 20–30°, and the residues were redissolved in 1.0 ml of benzene. An aliquot (100 μl) of each benzene fraction was placed in a glass culture tube, which then was placed in a polystyrene counting tube and assayed for radioactivity as described. The remainder of the benzene solution for each sample was stored overnight in a capped vial at 4°. The benzene then was evaporated, and the residues were redissolved in ether (10 drops) and spotted on TLC plates[6]. The vials were rinsed with another 10 drops of ether, which also was spotted on the plates. The plates then were developed once in *n*-hexane–ether–acetic acid (90:10:1) to 16 cm, air dried, and redeveloped in the same direction with *n*-hexane–ether–acetic acid (75:25:2). The plates were cut into 1-cm wide strips, starting 1 cm below the origin and continuing to the solvent front. Each strip was placed in a counting tube and assayed for radioactivity as described. Unlabeled 19-iodocholesterol, cholesterol, 19-iodocholesteryl palmitate, cholesteryl oleate, and pregnenolone were cochromatographed with the radioactive samples and visualized with iodine vapor to serve as reference standards.

Scintiscan of Female Rat—Two adult female Sprague–Dawley rats, 212 and 218 g, each received an intravenous injection of 245.4 μCi of ^{125}I-labeled 19-iodocholesterol in 0.7 ml of vehicle (Solution B) *via* the tail vein while under ether anesthesia. At 1 and 4 days postadministration, one rat was anesthetized with pentobarbital sodium by intraperitoneal injection (0.2 ml, 50 mg/ml) and scanned in the prone position using a small animal scanner. Following removal of the left ovary and adrenal gland, the rat was rescanned. The rat then was killed, and samples of the adrenal gland, ovary, and liver were assayed for radioactivity as described previously.

[5] Whatman No. 1.

[6] ITLC, Gelman, Ann Arbor, Mich.

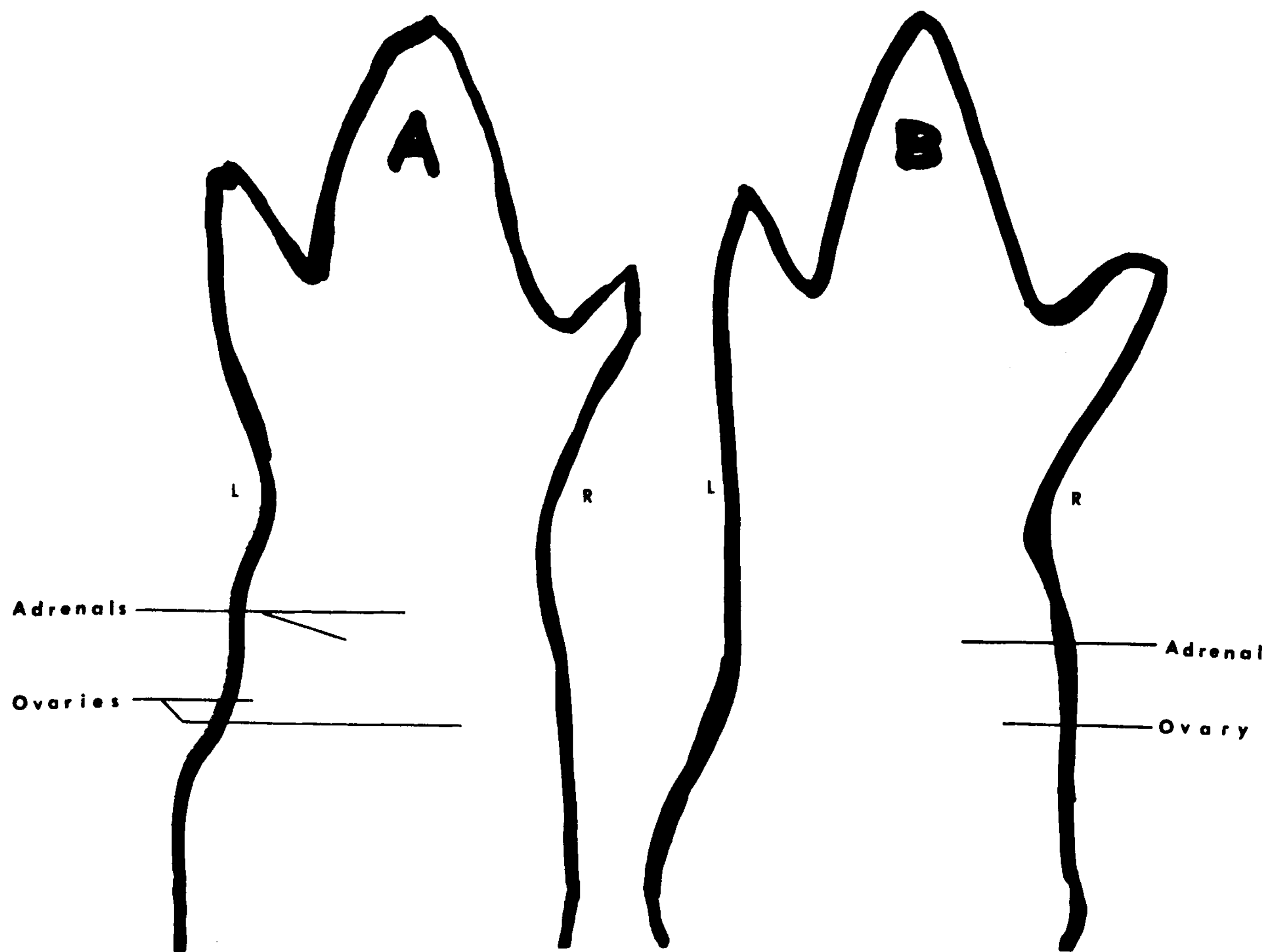

Figure 3—*Scintiscan of female rat 4 days after administration of ^{125}I-labeled 19-iodocholesterol (A) and rescan of the same rat following removal of the left ovary and left adrenal gland (B).*

RESULTS

Tissue Distribution—The distribution of radioactivity following intravenous administration of ^{125}I-labeled 19-iodocholesterol to female rats is summarized in Table I. High levels of radioactivity were noted in the adrenal cortex, ovaries, and thyroid at both times studied. The ovary to blood and ovary to liver ratios were 23 and 14 at 1 day postinjection and 138 and 47 at 4 days postinjection, respectively. The adrenal cortex to blood and adrenal cortex to liver ratios were 41 and 24 at the 1-day interval and 479 and 165 at the 4-day interval, respectively.

Folch Extraction—The results of the Folch extraction studies are summarized in Table II and Figs. 1 and 2. Greater than 90% of the radioactivity in the adrenal cortex was extractable into the lower chloroform phase at both the 1- and 4-day intervals. The remaining activity was found predominantly in the precipitated material, with only trace amounts in the upper aqueous phase. At the 1-day interval, 88% of the lower phase activity comigrated with the esterified standards; this activity increased to 95% by 4 days. While >90% of the radioactivity in the ovary also was extractable into the lower phase, only 58% of this activity comigrated with the esterified standards at the 1-day interval. Activity that comigrated with free sterol standards represented 25% of the lower phase activity. However, by 4 days, 86% of the activity was seen in the ester peak and only 10.4% was seen in the free sterol peak. Again, the remaining radioactivity was associated with the precipitated material.

For blood and liver samples, only 56–78% of the radioactivity was extractable into the lower phase. The remaining activity was found in both the precipitate and the upper phase. TLC analysis of the lower phase radioactivity showed that two distinct peaks were present; one peak corresponded to the free sterol standards, and one corresponded to the esterified sterol standards. However, unlike the adrenal gland and the ovary, most of the radioactivity was located in the free sterol peak.

Scintiscan of Female Rats—Attempts to scan the rat 1 day after administration of ^{125}I-labeled 19-iodocholesterol did not delineate the ovary or adrenal gland. Radioactivity present in the nontarget tissues of the abdominal cavity (*e.g.*, liver) as well as the adrenal glands and ovaries resulted in one large area of abdominal radioactivity. Removal of the left ovary and adrenal gland resulted in no detectable change in the image. Scintiscan of the rat 4 days after administration of ^{125}I-labeled 19-iodocholesterol (Fig. 3) resulted in the visualization of four areas of radioactivity. Removal of the left adrenal gland and ovary resulted in the corresponding disappearance of two radioactive areas when the rat was rescanned. The percent of dose per gram values for this animal were: adrenal, 10.707; ovary, 4.564; and liver, 0.062.

DISCUSSION

Results of tissue distribution and Folch extraction studies in rats showed that radioactivity was selectively accumulated and retained in an esterified form in the ovary as well as in the adrenal cortex following administration of ^{125}I-labeled 19-iodocholesterol. This finding is consistent with the reported similarities between the adrenal gland and ovary with regard to storage and utilization of cholesterol (3).

The retention of high levels of radioactivity in the adrenal gland and ovary several days after administration provides target to nontarget ratios necessary for resolution and visualization of these small organs. Although the level of radioactivity in the liver at 24 hr was considerably lower than that in either the adrenal gland or ovary, it was sufficient to interfere with imaging. However, after 4 days, the nontarget levels of radioactivity dropped significantly so that the target organs could be visualized.

The extremely high levels of radioactivity in the thyroid were noted previously (1) and are thought to be the result of *in vivo* deiodination and subsequent accumulation of free iodide in the thyroid where it becomes protein bound. Evidence for this conclusion lies in the blocking of thyroid

Table II—Percent of Radioactivity [a] Found in Each Phase following Folch Extraction of Tissue Radioactivity at 1 and 4 Days Postadministration of ^{125}I-Labeled 19-Iodocholesterol

Tissue	Day 1			Day 4		
	Precipitate	Upper	Lower	Precipitate	Upper	Lower
Adrenal	5.47 ± 2.36[b]	0.40 ± 0.42[b]	94.14 ± 2.48[b]	9.02 ± 3.54[b]	0.12 ± 0.14[b]	90.87 ± 3.65[b]
Blood	19.50 ± 4.74	13.87 ± 0.96	66.63 ± 5.21	29.00 ± 13.09	14.54 ± 4.98	56.47 ± 14.02
Liver	8.83 ± 2.00[b]	13.60 ± 2.04[b]	77.58 ± 2.29[b]	12.53 ± 1.91	11.18 ± 2.37	76.30 ± 3.87
Ovary	7.89 ± 2.21	0.92 ± 0.53	91.19 ± 1.82	8.81 ± 3.71	0.35 ± 0.10	90.84 ± 3.70

[a] Each value represents the mean ± *SD* for five rats unless noted. [b] Four rats.

uptake of radioactivity by pretreatment with potassium iodide solution (1) and in Folch extraction studies where >98% of the thyroid radioactivity was associated with the precipitated fraction[7].

The TLC systems used gave good separation of the free and esterified sterol. No further identification of radioactive products was attempted. Therefore, the esterified sterol peak could be made up of 19-iodocholesterol esterified with different saturated and unsaturated fatty acids. Similarly, slight alterations in the sterol portion of the molecule also could go undetected. Thus, [^{14}C]cholesterol and ^{125}I-labeled 19-iodocholesterol have the same R_f value in these systems.

Despite these limitations, these studies support the view that 19-iodocholesterol mimics cholesterol with regard to esterification and storage and that this property is a factor in its accumulation and retention for long periods in the adrenal cortex and ovary. In addition to its use in adrenal imaging, 19-radioiodinated cholesterol or related compounds may have clinical utility for the diagnosis of ovarian disorders.

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Allyl Derivatives of Zearalenone

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Abstract □ The Claisen rearrangement of 4-*O*-allylzearalenone led to only one principal product, which was identified as 3-allylzearalenone with the aid of photochemical isomerization and PMR spectroscopy. This general method is useful for distinguishing 3-substituted zearalenone isomers from 5-substituted isomers. Hydrogenation of 3-allylzearalenone gave 3-(1-propyl)zearalanone.

Keyphrases □ Zearalenone—allyl derivatives, synthesis □ Anabolic agents—zearalenone, synthesis of allyl derivatives

Zearalenone (I), a metabolite of *Gibberella zeae*, is produced *via* an industrial fermentation process (1). Several derivatives of I were synthesized to enhance its anabolic activity (2, 3). Zeranol (VI), a tetrahydro derivative, is used commercially as an anabolic agent in feedlot steers. The same fungus also produces four structurally related minor metabolites, one of which was characterized as 3-formylzearalenone (II) (4).

DISCUSSION

Isomeric 3- and 5-substituted zearalenones are not distinguished readily from each other by standard spectroscopic methods. Bolliger and Tamm (4) formylated zearalenone, and extensive PMR spectral scrutiny of the resulting 3- and 5-formylzearalenones and their corresponding dimethyl ethers led to the unequivocal assignment of Structure II to the metabolite (4). In another instance, III, the Kolbe–Schmitt reaction product of zearalenone, was subjected to extensive degradation (5).

This report describes a simple photochemical method coupled with PMR spectroscopy for a facile structural assignment. 4-*O*-Allylzearalenone (IV) was subjected to the Claisen rearrangement at 190–195°, and the principal product, V (mp 116–118°), was isolated in a 48% yield. It was isomerized to 3-allyl-*cis*-zearalenone (VIII) *via* the photochemical method of Peters (6). In the comparative PMR spectra of V and VIII, the upfield shift (13 Hz) of the aromatic proton of C-5 was very distinct, while the shifts of the allyl group protons were practically unchanged (6). Such an upfield shift of the aromatic proton of C-3 is not expected to occur in 5-substituted derivatives. The Claisen product thus was identified as 3-allylzearalenone (V). Low-pressure hydrogenation of VI gave 3-(1-propyl)zearalanone (VII).

EXPERIMENTAL[1]

4-*O*-Allylzearalenone (IV)—Compound I (15.9 g, 0.05 mole), allyl bromide (24.2 g, 0.2 mole), and potassium carbonate (10.0 g, 0.07 mole) were stirred and refluxed in acetone (200 ml) for 8 hr. The filtered inorganic salts were washed well with more solvent. The combined washings and filtrate were evaporated under reduced pressure. The residue was crystallized from 2-propanol to give 10 g of IV, mp 123–125°. In the PMR spectrum, a peak at δ 12.00 indicated the presence of the chelated hydroxyl proton at the 2-position; the absence of a peak at about δ 8.00 of the hydroxyl proton at the 4-position indicated allylation of this site. With two aromatic protons at δ 6.38 and 6.48 (J = 2.5 Hz), the rest of the spectrum was consistent with the assigned structure.

[1] Melting points (uncorrected) were measured with a Thomas-Hoover apparatus. PMR spectra were recorded on a Varian A 60-A instrument in deuterochloroform with tetramethylsilane as the internal standard. Elemental analyses were carried out by Microanalyses Inc.

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