observed with other excipients studied in this investigation exhibit smaller changes, the spectral and color differences observed were indicative of some interaction. The diversity of the excipient or other type materials found in Table II also indicates that this ferrous sulfate interaction is widespread.

It should therefore be recognized that although analytical data with respect to a dosage form may substantiate the presence of the labeled amount of metallic ion, the form in which it exists in this product may certainly be one that is not available for a therapeutic response (11). Based on this ferrous sulfate-excipient interaction data, it does not seem unreasonable that other iron salts as well as salts used for other therapeutic purposes would also undergo such a phenomenon. A consideration of this possibility is therefore necessary in the development of pharmaceutical products.

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Keyphrases

Iron—adjuvant interactions
Ferrous sulfate-adjuvant reactions—aqueous dispersion
Diffuse reflectance spectroscopy—analysis

Tumor Localizing Agents VI

Radioiodinated Analogs of Dichlorodiphenyldichloroethane (DDD)

By R. E. COUNSELL, V. V. RANADE, P. POCHA, R. E. WILLETTE, and W. DiGUILIO

The preparation of 1,1-dichloro-2-(*p*-chlorophenyl)-2-(*o*-iodophenyl-^{125}I)ethane and 1,1-dichloro-2-(*p*-chlorophenyl)-2-(*m*-iodophenyl-^{125}I)ethane is reported. These compounds were prepared by chlorination of the appropriate iodoacetophenone, reduction to the corresponding carbinol, followed by acid-catalyzed condensation with chlorobenzene. Radioiodination was accomplished by isotope exchange with iodide-125. Preliminary studies in rats indicate that the position of the radioiodine has little influence on the predilection of these agents for adrenal tissue.

Wolf and Tubis (1) recently reviewed the rapidly expanding field of radiopharmaceuticals. As noted by these workers, various gamma-emitting radionuclides are now employed to externally scan many organs and major parts of the body.

Scanning has become a valuable diagnostic technique and permits the visualization of an internal organ by outlining the distribution of a radionuclide concentrating within an organ or tumor. With this technique, iodine-131 has been used to localize tumors of the thyroid, and chlormerodrin labeled with mercury-197 has served to delineate brain and kidney tumors. To date, however, no radiopharmaceutical is available for photoscanning the adrenal gland, although several laboratories are currently in the process of attempting to develop such an agent.

Nagai *et al.* (2) have reported that ^{131}I-labeled stigmasterol was useful in some cases for adrenal scanning. The agent was not regularly successful, however, because of the uptake in the liver and the excretion of free ^{131}I in the stomach. Similarly, because human adrenal glands con-

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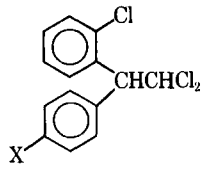
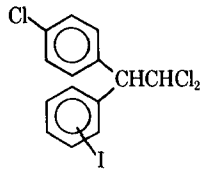
Part V, Counsell, R. E., Hong, B. H., Willette, R. E., and V. V. Ranade, *Steroids*, *11*, 817(1968).

tain a high concentration of ascorbic acid, Mishkin and Castronovo (3) evaluated ^{14}C-labeled ascorbic acid as a possible lead to an adrenal scanning agent. Unfortunately, their studies revealed that the liver interfered once again and concentrated approximately 35 times more ascorbic acid than did the adrenal.

Previous reports from this laboratory (4) have indicated that certain radioiodinated analogs of 1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane (*o,p'*-DDD, I*a*) may offer some promise for adrenal photoscanning in humans. For example, preliminary studies with 1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p*-iodophenyl)-ethane-^{125}I (I*b*) showed that the general order of concentration 4 hr. following administration to rats was adrenal > fat > liver and other tissues. Additional preclinical studies in dogs are currently in progress with this agent.



I*a*, X = Cl
b, X = I

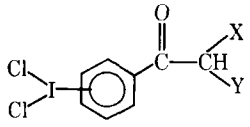


II*a*, *o*-iodo
b, *m*-iodo
c, *p*-iodo

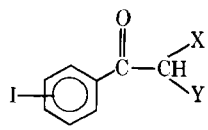
The author's studies with radioiodinated DDD analogs were prompted by the fact that *o,p'*-DDD causes adrenal atrophy in dogs (5) and is currently the treatment of choice for adrenocortical carcinoma (6). It apparently exerts its antitumor action by concentrating in adrenal tissue (7). Interestingly, isomeric *p,p'*-DDD was found to be essentially devoid of adrenolytic activity in dogs (5).

In an effort to obtain further information regarding structure and adrenal localization, this paper describes the synthesis and compares the tissue distribution of additional radioiodinated DDD analogs in which the position of the aromatic iodine has been varied (II).

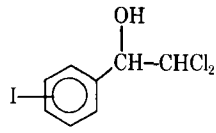
The preparation of the *p,p'*-isomer, II*c*, was reported previously (4) and involved the acid-catalyzed condensation of iodobenzene with 2,2-dichloro-1-(*p*-chlorophenyl)ethanol. For the preparation of II*a* and *b*, the *ortho* and *meta* isomers of 2,2-dichloro-1-(iodophenyl)ethanol (VIII) were required. These compounds were prepared by side chain chlorination of the appropriate iodoacetophenone and subsequent reduction to the carbinol with lithium tri-*t*-butoxyaluminum hydride.



III X = Y = H
IV X = Cl, Y = H
V X = Y = Cl



VI X = Cl, Y = H
VII X = Y = Cl



VIII

a, *o*-iodo; *b*, *m*-iodo

The chlorination of the iodoacetophenones by methods previously employed for the chloroacetophenones was complicated by the ability of aryl iodides to readily add 1 mole of chlorine to form dichlorides (8). For example, when chlorine gas was passed into an acetic acid solution of *m*-iodoacetophenone, the internal temperature quickly rose to 50° and a yellow precipitate of the dichloride (III*b*) formed immediately. An examination of the filtrate afforded a low yield of the chloroketone, VI*b*. When chloroform was used as the solvent, III*b* was obtained exclusively. If chlorination was continued at 50–60°, the yellow dichloride gradually dissolved and quenching the reaction at this stage furnished the chloroketone VI*b* in good yield. If chlorination was conducted above 70°, a second yellow precipitate formed which was the dichloride of the dichloroacetophenone, V*b*. The dichlorides are unstable and liberate chlorine on heating to give the appropriately substituted iodoacetophenones. Thus, the dichloroketone VII*b* was readily obtained by heating V*b* to 150°. No nuclear chlorination was observed to occur.

Chlorination of *o*-iodoacetophenone at 70°, on the other hand, gave rise to the dichloride of the trichloroketone. This product rapidly lost chlorine upon heating to afford 2,2,2-trichloro-2'-iodoacetophenone. This structure was established on the basis of elemental analysis, the presence of only aromatic protons in the NMR, and its quantitative conversion to *o*-iodobenzoic acid upon treatment with aqueous sodium hydroxide. The desired dichloroketone VII*a* was obtained in excellent yield by a slight modification of the chlorination conditions.

The carbinols (VIII*a* and VIII*b*) obtained after reduction of the halogenated acetophenones with lithium tri-*t*-butoxyaluminum hydride were condensed with chlorobenzene. The resulting

TABLE I—TISSUE/LIVER RATIOS OF RADIOACTIVITY AT 4 hr. IN RATS

Compd.-^{125}I	Adrenal	Perirenal Fat	Serum	Muscle	Kidney
I*b*	1.66 (0.12)[a]	2.11 (1.38)	0.66 (0.47)	0.48 (0.41)	0.55 (0.22)
II*a*	0.74 (0.09)	0.99 (0.20)	0.42 (0.24)	0.28 (0.06)	0.24 (0.01)
II*b*	1.47 (0.57)	1.33 (0.40)	0.41 (0.15)	0.39 (0.14)	0.44 (0.10)
II*c*	2.22 (0.48)	2.01 (1.05)	0.32 (0.04)	0.34 (0.16)	0.42 (0.07)

[a] Standard deviation in parentheses.

iodinated analogs of DDD were then subjected to isotope exchange with iodide-125 under conditions similar to those previously reported from this laboratory (4).

Tissue Distribution Studies—Preliminary tissue distribution data in male rats are summarized in Table I. The values are expressed as a ratio of the concentration of radioactivity found in a particular tissue to that for liver. Since the liver is the nearest interfering organ in adrenal scanning, it is essential that a compound exhibit a high adrenal/liver ratio in order to be considered for follow-up studies.

In contrast with results in the DDD series (6, 7), the radioiodinated analogs did not differ greatly in their capacity to concentrate in adrenal tissue. In fact, these preliminary results would tend to indicate that the *p,p'*-isomer (II*c*) has the greatest specificity for adrenal tissue. In all cases, the concentration of radioactivity in fat increased progressively and was three to four times higher than the adrenal content at 24 hr. More detailed studies in rats and additional studies in dogs are now in progress and will be reported elsewhere.

EXPERIMENTAL[1]

Chlorination of o-Iodoacetophenone—*Method A*—A solution of *o*-iodoacetophenone[2] (5 g.) in acetic acid (30 ml.) was treated with Cl_2 gas at 50–55°. Nitrogen was then passed through the reaction mixture to remove excess chlorine and hydrogen chloride. The temperature was raised to 60–65° and Cl_2 passed into the solution for 3.5 hr. Excess Cl_2 was swept out with N_2 as before and the solution allowed to cool. The solution was then poured into water (300 ml.) containing sodium sulfate (2 g.) and sodium acetate (6 g.). The mixture was extracted with ether and the ether extract was washed well with water and dried (Na_2SO_4). The solvent was removed and the residual oil distilled *in vacuo*. This gave pure VII*a* (6 g., 87%) as a yellow mobile liquid, b.p. 112–114° (0.2 mm.), $\nu_{max.}$ 3010, 2950, 1280, and 750 cm.$^{-1}$. The NMR spectrum showed a single peak at 6.64 δ (—$CHCl_2$).

Anal.—Calcd. for $C_8H_5Cl_2IO$: C, 30.52; H, 1.60. Found: C, 30.66; H, 1.57.

Method B—Chlorine gas was passed into a solution of *o*-iodoacetophenone (2 g.) in acetic acid (15 ml.). A yellow precipitate formed immediately and the temperature of the reaction mixture increased to 55°. The gas flow was stopped and the reaction mixture heated until the solid dissolved (65–70°). The gas flow was started and continued for 30 min. The mixture was refrigerated and the yellow precipitate collected (2.7 g.), m.p. 73–90° with evolution of Cl_2. This product was heated in an oil bath (130°) until gas evolution stopped and the residual oil distilled. This gave 2 g. of a pale yellow oil, b.p. 108–110° (0.25 mm.). The NMR spectrum displayed only aromatic protons. Elemental analysis of this product and its quantitative conversion to *o*-iodobenzoic acid upon treatment with aqueous NaOH established the product as 2,2,2-trichloro-2'-iodoacetophenone.

Anal.—Calcd. for $C_8H_4Cl_3IO$: C, 27.50; H, 1.15. Found: C, 27.66; H, 1.34.

Chlorination of m-Iodoacetophenone — *Method A*—Chlorine gas was bubbled through a solution of *m*-iodoacetophenone[2] (5 g.) in acetic acid (15 ml.). The temperature of the reaction rose to 50° and a yellow precipitate began to form immediately. Filtration and washing with water gave the dichloride of starting material (III*b*, 7 g.), m.p. 100° (from $CHCl_3$) with evolution of Cl_2 and $\nu_{max.}$ 1680 cm.$^{-1}$ (C=O).

Anal.—Calcd. for $C_8H_7Cl_2IO$: C, 30.31; H, 2.23; Cl, 22.37; I, 40.03. Found: C, 30.49; H, 2.28; Cl, 22.09; I, 40.21.

The compound was unstable to heat and light and lost Cl_2 on standing at room temperature, to yield *m*-iodoacetophenone. The mother liquors from the reaction were poured into ice water and extracted with ether, dried (Na_2SO_4), and evaporated to give the monochloroacetophenone, VI*b*. Recrystallization from CH_2Cl_2-hexane gave pure VI*b* (0.1 g.), m.p. 69–70°, $\nu_{max.}$ 1710 cm.$^{-1}$ (C=O). The NMR spectrum showed a sharp singlet at 4.64 δ integrating for two protons.

Anal.—Calcd. for C_8H_6ClIO: C, 34.25; H, 2.16. Found: C, 34.07; H, 2.08.

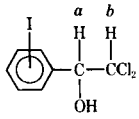
When the reaction was repeated using dry chloroform as solvent instead of acetic acid, the dichloride (III*b*) was formed exclusively.

Method B—Chlorine gas was passed through a solution of *m*-iodoacetophenone (5 g.) in acetic acid (50 ml.). A yellow precipitate formed immediately but dissolved upon heating the reaction mixture to 70°. Chlorination was continued at this temperature until yellow needles began to

[1] 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. Infrared spectra were taken in KBr disks on a Perkin-Elmer 337 spectrophotometer. The NMR spectra were obtained with a Varian A-60 spectrometer in $CDCl_3$ at a concentration of 10%, with tetramethylsilane as internal reference. Thin-layer chromatograms (TLC) were run with 1 in. wide Eastman Chromagrams, type K301R with fluorescence indicator, developed with benzene, and spots detected with UV light and iodine vapor. Chromagrams were scanned with an Atomic Associates RCS-363 Radiochromatogram scanner. The specific activities were determined with a Beckman scintillation spectrometer model 530.

[2] Prepared according to Bowman, R. E., *J. Chem. Soc.*, 1950, 322.

TABLE II—CHEMICAL DATA FOR



Compd.	B.p., °C.(mm.)	Yield, %	Anal.[a] C	Anal.[a] H	Chemical Shifts (δ) H(a)	Chemical Shifts (δ) H(b)	J_{AX}, c.p.s.
VIII*a*	115–117 (0.2)	62	30.47	2.37	5.31	6.06	3.5
b	123–124 (0.12)	35	30.40	2.35	4.90	5.80	5

[a] *Anal.*—Calcd. for $C_8H_7Cl_2IO$: C, 30.29; H, 2.21.

separate (20 min.). The reaction mixture was allowed to cool and the product was collected by filtration. The product was washed with dry ether to give the dichloride of the α-dichloroketone (V*b*, 5.5 g.), m.p. 97–115°. Recrystallization of a sample from benzene gave pure V*b*, m.p. 125–127°, which was extremely unstable and promptly lost Cl_2. The remaining crude V*b* was heated to 150° until the evolution of gas ceased. The residual oil was distilled *in vacuo* to afford the dichloroacetophenone, VII*b* (2.7 g.), b.p. 108° (0.1 mm.), $\nu_{max.}$ 1725 cm.$^{-1}$ (C=O). The NMR showed a singlet at 5.04 δ (—COCH—) integrating for one proton.

Anal.—Calcd. for $C_8H_5Cl_2IO$: C, 30.51; H, 1.60. Found: C, 30.50; H, 1.77.

Method C—A solution of *m*-iodoacetophenone (10 g.) in acetic acid (35 ml.) was warmed to 50° and Cl_2 gas passed through. The yellow precipitate that formed initially was dissolved by warming to 60° and Cl_2 gas passed into the reaction mixture for an additional 2 hr. The warm reaction mixture was then poured onto ice whereupon a white solid formed. This was extracted with benzene, dried ($MgSO_4$), and evaporated to give a colorless oil which crystallized on refrigeration. The solid was collected and washed with benzene–pentane to give 2-chloro-3′-iodoacetophenone (VI*b*) as white needles (6.8 g.), m.p. 53–58°. Recrystallization of 0.5 g. from CH_2Cl_2–hexane afforded 0.2 g. as silky needles, m.p. 69–70°. In addition to aromatic protons, the NMR showed a singlet at 4.63 δ (—CH_2Cl).

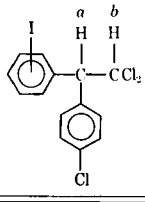
Anal.—Calcd. for C_8H_6ClIO: C, 34.25; H, 2.16. Found: C, 34.07; H, 2.08.

Reduction of Chlorinated Iodoacetophenones—*General Method*—A solution of the dichloroketones (VII, 20 m*M*) in tetrahydrofuran (20 ml.) was added dropwise with stirring to a solution of lithium tri-*t*-butoxyaluminum hydride (30 m*M*) in tetrahydrofuran (40 ml.). The mixture was stirred at room temperature for 2–5 hr. and poured into ice water (500 ml.) containing acetic acid (25 ml.). The mixture was extracted with chloroform, the extract dried ($MgSO_4$), and the solvent removed *in vacuo*. The residual pale yellow oils were fractionally distilled to give the corresponding carbinols, VIII (see Table II).

Condensation of VIII with Chlorobenzene—*General Method*—To a solution of VIII (1 g.) in chlorobenzene (3 ml.) was added dropwise with stirring a saturated solution of boron trifluoride in sulfuric acid. The addition required 30 min. whereupon the mixture was heated at 40–45° for 4 hr. The mixture was extracted with benzene, and the extract washed with water and dried ($MgSO_4$). The solvent was removed *in vacuo* and the oily residue triturated with petroleum ether (30–40°). The resulting solid was collected by filtration and recrystallized from 95% EtOH (see Table III).

Isotope Exchange—*General Method*—A solution containing 3 mc. of $Na^{125}I$ was placed in a 10-ml. round-bottom flask and evaporated to dryness at 100° under a gentle stream of N_2. Ethylene glycol (1 ml.) was added, the external temperature raised to 175–180°, and the iodinated compound (II) added. The solution was magnetically stirred for 5 hr., cooled, and diluted with water. The mixture was extracted with benzene and the extract washed with water, dried ($MgSO_4$, Darco), and concentrated to dryness. The products were purified by recrystallization from 95% ethanol and the purity

TABLE III—CHEMICAL DATA FOR



Compd.	M.p., °C.	Yield, %	Anal.[a] C	Anal.[a] H	Chemical Shifts (δ) H(a)	Chemical Shifts (δ) H(b)	J_{AX}, c.p.s.
II*a*	104–105	62	40.70	2.52	5.05[b]	6.29	8
b	87.5–88.5	31	40.95	2.50	4.48	6.27	8

[a] *Anal.*—Calcd. for $C_{14}H_{10}Cl_2I$: C, 40.87; H, 2.45. [b] The deshielding effect of *ortho*-substituted halogens on benzylic protons has been noted previously (9).

TABLE IV—ISOTOPE EXCHANGE FOR COMPOUNDS II

Compd.	Recovery, %	Exchange, %	Spec. Act., mc./mg.
IIa	10	15	5.22
b	70	62.8	17.58

established by (*a*) melting point and (*b*) TLC and a radiochromatogram of the strip (see Table IV).

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Keyphrases

Tumor localizing agents—synthesis
Dichlorodiphenyldichloroethane analogs—radioiodinated
Radioiodination—iodide-125
Tissue distribution—radioiodinated dichlorodiphenyldichloroethane analogs

Interrelationship of Phosphate Nutrition, Nitrogen Metabolism, and Accumulation of Key Secondary Metabolites in Saprophytic Cultures of *Psilocybe cubensis, Psilocybe cyanescens,* and *Panaeolus campanulatus*

By J. M. NEAL, R. G. BENEDICT, and L. R. BRADY

The three basidiomycetes were grown on rotary shakers in four nutrient media containing various amounts of phosphate to determine the relative effect of this nutrient on the trichloroacetic acid-soluble and -insoluble nitrogen metabolites and to detect possible correlation between the fungal free amino acid pool (soluble nitrogen) and accumulation of characteristic hydroxytryptamine derivatives. The species were selected to represent different patterns of metabolism in fruiting bodies and vegetative mycelia. Vegetative mycelium of *P. cubensis* was characterized by a relatively high soluble nitrogen component and by the capacity to accumulate psilocybin and psilocin under selected conditions. The closely related *P. cyanescens* was less responsive to variations in phosphate nutrient and lacked the capacity to accumulate appreciable quantities of soluble nitrogenous compounds or detectable quantities of key tryptamines. *P. campanulatus* grown in phosphate-rich media appeared to have an adequate free amino acid pool and to excrete some exocellular nitrogen metabolites during longer incubation periods; no 5-hydroxytryptamine derivatives were detected in cultures of this fungus.

OBSERVATIONS on the occurrence of various secondary metabolites in basidiomycetes have demonstrated the medicinal interest in certain of these fungi (1). However, extensive investigation or utilization of many of the empiric observations has been delayed by the lack of basic biologic knowledge which would permit manipulation of the basidiomycetes in the manner of antibiotic-producing actinomycetes or various commercially useful ascomycetes. Information on the occurrence of many basidiomycete metabolites suggests that fungal fruiting bodies and

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