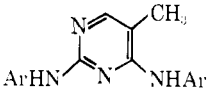
TABLE I
2,4-BIS(ARYLAMINO)-5-METHYLPYRIMIDINES



Compd	Ar	Yield, % (crude)	Reaction time, hr	Mp, °C[a]	Solvent of recrystn	Formula	Caled	Found	Caled	Found	Caled	Found
I	C_6H_5	82	1.5	175–177	Aq ethanol	$C_{17}H_{16}N_4$	73.91	73.71	5.83	6.00	20.28	20.08
II	p-$NO_2C_6H_4$	85	0.5	>300	DMF	$C_{17}H_{16}N_6O_4$	55.73	55.59	3.82	4.01	22.95	22.78
III	p-ClC_6H_4	92	1	215–217	Aq ethanol	$C_{17}H_{14}Cl_2N_4$	59.15	59.20	4.05	4.15	16.23	16.00
IV	m-ClC_6H_4	86	1	183	Aq ethanol	$C_{17}H_{14}Cl_2N_4$	59.15	58.99	4.05	4.19	16.23	16.43
V	o-ClC_6H_4	80	1	142–143	Aq ethanol	$C_{17}H_{14}Cl_2N_4$	59.15	59.20	4.05	4.30	16.23	15.98
VI	p-OHC_6H_4	77	6	>300	1 N HCl	$C_{17}H_{16}N_4O_2 \cdot HCl$	59.50	59.42	4.60	4.73	16.22	16.00
VII	p-$CH_3C_6H_4$	90	1	170	Aq ethanol	$C_{18}H_{20}N_4$	75.00	75.10	6.57	6.70	18.42	18.36
VIII	p-$OCH_3C_6H_4$	70	2	180–183	Aq ethanol	$C_{18}H_{20}N_4O_2$	67.85	68.10	5.95	5.99	16.60	16.72
IX	p-$COCH_3C_6H_4$	70	3	>300	1 N HCl	$C_{21}H_{20}N_4O_2 \cdot HCl$	60.30	60.22	5.04	5.24	14.19	14.00

[a] All melting points were determined in capillary tubes in a Gallenkamph apparatus and are corrected.

Crystals began to appear within 15–20 min. The refluxing was stopped after 1 hr and the reaction mixture was kept overnight in a refrigerator. The crystalline product was filtered off and washed with cold water. It was then suspended in about 30 ml of water, neutralized with dilute NH_4OH, cooled, and collected by filtration as free base. This compound could be easily recrystallized from 80% ethanol.

The other compounds listed in Table I were synthesized by the same general method. As indicated in Table I the time of refluxing had to be extended in certain cases. Some compounds were recrystallized as hydrochlorides since they could not be satisfactorily crystallized as free bases. All compounds were recrystallized from suitable solvent and were dried *in vacuo* at 110° for 24 hr before analysis.

Inhibition of Growth of Microorganisms.—All compounds were tested for their antimicrobial activity against *Streptococcus faecalis*, *Escherichia coli* B, *Salmonella typhimurium*, and a pathogenic strain of yeast, *Candida albicans*. The concentrations of synthetic compounds necessary for 50% inhibition of growth were determined turbidimetrically by serial dilution technique in test tubes using liquid growth medium[1b] (shown in Table II).

TABLE II
ANTIMICROBIAL ACTIVITIES OF
2,4-BIS(ARYLAMINO)-5-METHYLPYRIMIDINES

Compd	Concn for 50% inhib of growth, μg/ml — *S. faecalis*	*E. coli* B	*S. typhimurium*	*C. albicans*
I	2.15	1.96	1.60	9.00
II	*a*	*a*	*a*	*a*
III	1.30	1.00	0.85	0.92
IV	2.20	1.12	0.95	1.06
V	2.40	1.30	1.00	2.60
VI	81.80	102.00	22.00	51.00
VII	1.80	1.32	0.90	4.30
VIII	2.00	1.80	1.00	9.60
IX	1.95	8.50	2.00	23.50
6-Azauracil	12.00	7.20	5.60	*b*
Neomycin	*b*	1.30	1.55	1.10
Chloramphenicol	1.50	1.00	0.66	*b*

[a] Could not be tested due to low solubility in the common solvents. [b] Little or no activity.

Acknowledgment.—The authors wish to thank Dr. D. M. Bose, Director, Bose Institute, for his interest in this work. Thanks are also due to Dr. A. Sen for helpful suggestions and to Mrs. C. Dutta for microanalyses. One of the authors (M. M.) is a Fellow of Council of Scientific and Industrial Research, Government of India.

Tumor Localizing Agents. II. Radioiodinated Analogs of 1,1-Dichloro-2,2-bis(chlorophenyl)ethane

R. E. COUNSELL, R. E. WILLETTE, AND W. DIGUILIO

College of Pharmacy and Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan 48104

Received March 15, 1967

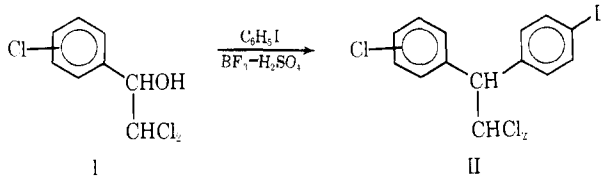
Wagner[1] has reviewed the important role currently being played by radioactive pharmaceuticals as diagnostic agents in clinical medicine. With the advent of a number of radiopharmaceuticals and the development of accurate detection instruments, it is now possible to externally scan most organs and major parts of the body. To date, however, no agent has been found which is suitable for photoscanning the adrenal gland and its associated tumors. As part of a broad program aimed at the development of a radiopharmaceutical which may be of value in the diagnosis and therapy of adrenal tumors, we wish to report on the synthesis and tissue distribution of some radioiodinated analogs of 1,1-dichloro-2,2-bis(chlorophenyl)ethane (DDD).

Our interest in structures related to DDD was prompted by the many reports in the literature indicating a predilection of these substances for adrenal tissue.[2] Nelson and Woodard[3] observed that commercially available technical *p,p'*-DDD caused necrosis of certain regions of the adrenal cortex in dogs. Some years later, however, two groups found that the adrenocorticolytic action of the commercial product was actually due to the *o,p'* isomer present as a contaminant.[4,5] Since that time, *o,p'*-DDD has been the subject of a number of biological and clinical investigations and these have been recently reviewed by Nichols.[2] Further stimulus to the study of *o,p'*-DDD and related compounds was provided when this substance was found to produce tumor regression in cases of metastic adrenal cortical carcinoma[6] and to cause remission of symptoms in patients with Cushing's syndrome.[7]

(1) H. N. Wagner, *Clin. Pharmacol. Therap.*, **4**, 351 (1963).
(2) *Cf.* J. Nichols in "The Adrenal Cortex," H. D. Moon, Ed., P. B. Hoebner, Inc., New York, N. Y., 1961, p 84.
(3) A. A. Nelson and G. Woodard, *Arch. Pathol.*, **48**, 387 (1949).
(4) J. Nichols and G. Hennigar, *Exptl. Med. Surg.*, **15**, 310 (1957).
(5) C. Cueto and J. H. V. Brown, *Endocrinology*, **62**, 324 (1958).

In the development of a radiopharmaceutical for diagnostic purposes, it is not only important that the agent localize within the organ or tumor desired (*i.e.*, high target:nontarget ratio) but also that the radiation emitted by the agent be readily detected by external detecting equipment. For this purpose, radionuclides with a γ-radiation energy of less than 500 kev are preferred.[8] Since the useful radioactive isotopes for carbon, hydrogen, and chlorine are all β emitters, another element had to be used for the present study. Iodine has two γ-emitting radioisotopes useful for radiolabeling purposes, ^{125}I and ^{131}I. Iodine-125 was selected for our preliminary studies because its longer half-life (60 *vs.* 8 days) and lower radiation energy (35 *vs.* 360 kev) simplified the synthesis and storage of the products.

In a previous publication,[9] we reported on the synthesis of ^{14}C-labeled *o,p'*-, *m,p'*-, and *p,p'*-DDD. As a follow-up to this work, the corresponding radioiodinated analogs (II) in which the *p*-chlorine was replaced by ^{125}I were desired. Acid-catalyzed condensation of iodobenzene with the appropriate isomer of 2,2-dichloro-1-(chlorophenyl)ethanol (I) gave the desired iodo analogs in good yield. The synthesis of the



I II

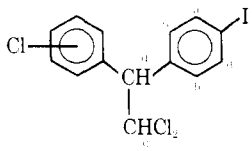
a, *o*-Cl; b, *m*-Cl; c, *p*-Cl

starting dichloroethanols (I) was reported on previously.[9]

Comparison of the uv spectra of these iodo analogs with the corresponding DDD isomer showed the bathochromic shift (12 ± 1 mμ) of the E band and enhancement in molar absorptivity expected when an aromatic chlorine is replaced with iodine.[10] As previously noted for the isomeric DDD's,[9] the nmr spectrum for the *ortho* product IIa was readily distinguished from IIb and IIc by the downfield shift of the benzylic proton by 39 ± 1 cps (see Table I). The aromatic protons on the ring bearing iodine displayed the typical $A_2'B_2'$ pattern for all three isomers with a J_{ax} = 8.5 cps. The aromatic protons for the chlorinated ring, on the other hand, appeared as a singlet at 7.22 and 7.26 ppm for IIb and IIc and as a multiplet centered at approximately 7.20 ppm for IIa.

TABLE I

CHEMICAL SHIFTS OF DDD ANALOGS IN $CDCl_3$



Isomer	Chemical shift, ppm— H_a	H_b	H_c	H_d	J_{ax}, cps
IIa	7.64	7.10	6.35	5.16	8.5
IIb	7.65	7.03	6.27	4.47	8.2
IIc	7.66	7.05	6.29	4.52	7.5

Isotope exchange was employed to introduce ^{125}I into the isomeric iodo compounds. This was best achieved by heating the appropriate isomer with $Na^{125}I$ in ethylene glycol at 180°. Under these conditions, approximately 50% exchange was usually achieved within 12 hr. The rate of exchange was readily followed by radioscanning thin layer chromatograms (tlc) taken at appropriate time intervals. Dimethyl sulfoxide (DMSO) was also evaluated as an exchange media but found to give rise to a radioactive by-product which was difficult to separate from the desired product.

Tissue Distribution.—Preliminary tissue distribution data for the three radioiodinated analogs of DDD have been obtained in male rats. The compounds were administered in DMSO by intravenous injection. The general order of concentration at 4 hr was adrenal > fat > liver and other tissues. By 24 hr, the order was found to be fat > adrenal > liver and other tissues. Additional studies with these compounds are in progress and the results will be reported elsewhere.

Experimental Section[11]

Synthesis of Isomeric 1,1-Dichloro-2-(chlorophenyl)-2-(*p*-iodophenyl)ethanes (II). General Method.—A mixture of the appropriate 2,2-dichloro-1-(chlorophenyl)ethanol (2.26 g, 0.01 mole) and iodobenzene (2.04 g, 0.01 mole) was stirred at 40° and BF_3-saturated H_2SO_4 (6 ml) was added dropwise over 5 min. The mixture was heated at 40° with vigorous stirring for 3 hr and extracted with petroleum ether (bp 30–40°). The extract was washed with water and dried ($MgSO_4$ and charcoal), and the solvent was removed by distillation. The oily residue usually crystallized upon trituration with a little hexane but sometimes refrigeration was necessary. The product was collected by filtration and recrystallized from either methanol or 95% ethanol (see Table II).

TABLE II

Isomer	% yield	Mp, °C	$\lambda_{max}^{C_2H_5OH}$, mμ	Log ε	Found, % C	H
IIa	77	101–102	240.5	4.35	40.49	2.37
IIb	71	82.5–83.5	240	4.43	41.04	2.50
IIc	52	120–122	239.5	4.39	40.86	2.50

Anal. Calcd for $C_{13}H_9Cl_3I$: C, 40.86; H, 2.45.

Isotope Exchange. General Method. An aliquot of carrier-free $Na^{125}I$[12] in ethylene glycol (reagent grade) was diluted to the desired volume with more glycol and the isomer to be exchanged was added. The stirred suspension was brought to 180° and heating was continued for the period indicated (see Table III). The solution was cooled in ice and diluted with an equal volume of water. The mixture was extracted with benzene (seven 3-ml portions) and the extract was dried ($MgSO_4$ and

(6) D. M. Bergenstal, R. Hertz, M. B. Lipsett, and R. H. Moy, *Ann. Internal Med.*, **53**, 672 (1960).

(7) T. S. Danowski, M. E. Sarver, C. Moses, and J. V. Bonessi, *Am. J. Med.*, **37**, 235 (1964); D. A. D. Montgomery and R. B. Welbourn, *Brit. Med. J.*, **2**, 1356 (1965).

(8) H. N. Wagner, *Nucleonics*, **24**, 62 (1966).

(9) R. E. Counsell and R. E. Willette, *J. Pharm. Sci.*, **55**, 1012 (1966).

(10) A. E. Gillam and E. S. Stern, "Electronic Absorption Spectroscopy," 2nd ed, Edward Arnold Ltd., London, 1957, p 140.

(11) 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. Ultraviolet spectra were recorded on a Beckman DK2A spectrophotometer in 95% ethanol. The nmr spectra were obtained with a Varian A-60 spectrometer in $CDCl_3$ at a concentration of 10%, with Me_4Si as internal reference. Thin layer chromatograms (tlc) were run with 1-in. wide Eastman Chromagrams, Type K301R, with fluorescenre indicator, developed with benzene, and spots detected with uv light and iodine vapor. Chromagrams of radioiodinated compounds were scanned with an Atomic Associates RCS-363 radiochromatogram scanner. The specific activities were determined with an Atomic Associates well scintillation counter Model 810C and scintillation spectrometer Model 530.

(12) Obtained from Nuclear Science and Engineering Corp., Pittsburgh, Pa., in basic sodium sulfite solution.

charcoal) and evaporated. The residue was taken up in a minimum amount of 95% ethanol and cooled. The crystalline product was collected and recrystallized repeatedly until only a single radioactive spot could be seen after radioscanning a tlc strip.

TABLE III

Isomer	Amt used, mg	Reaction time, hr	% recovery	% exchange	Spec act., μcuries/mg
IIa	450	12	51	50.9	9.95
IIb	450	15	42	52.4	9.13
IIc	500	9.5	63	26.7	4.98

Acknowledgment.—The authors are grateful to Dr. William H. Beierwaltes for his interest and encouragement during the course of this work. Support for this investigation was provided by Grants CA-08349-01 from the National Cancer Institute, U. S. Public Health Service, Bethesda, Md., and PRA-18 from the American Cancer Society, New York, N. Y. Grants from the Horace H. Rackham School of Graduate studies and the Cancer Research Institute of the University of Michigan contributed to the initiation of this study.

Tumor Inhibitors. XXV. The Synthesis and Evaluation of 9-Nitro-1,2,3,4-tetrahydrophenanthrene-8-carboxylic Acid[1]

S. MORRIS KUPCHAN AND MICHAEL MOKOTOFF[2]

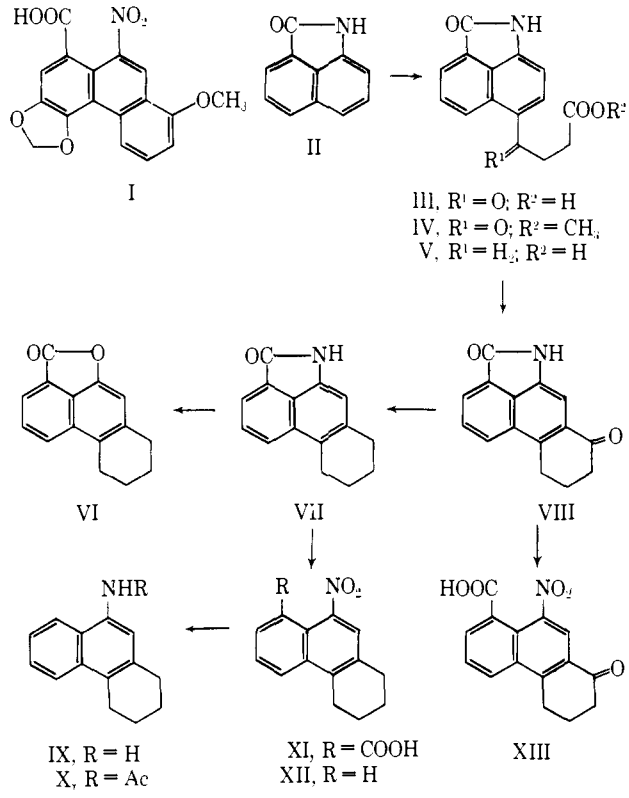
Department of Pharmaceutical Chemistry, University of Wisconsin, Madison, Wisconsin

Received April 7, 1967

In the course of a continuing search for tumor inhibitors of plant origin, aristolochic acid (I) was characterized as a tumor (Adenocarcinoma 755)-inhibitory principle from *Aristolochia indica* L.[3] A subsequent report described a synthetic approach to aristolochic acid and related phenanthrene carboxylic acids.[4] We report herewith the synthesis and evaluation of an aristolochic acid analog without oxygen ether functions and with a saturated ring, namely 9-nitro-1,2,3,4-tetrahydrophenanthrene-8-carboxylic acid (XI).

Naphthostyril (8-amino-1-naphthoic acid lactam, II) proved to be a useful starting material for a Haworth synthesis of XI (see Scheme I). In accord with expectation based upon analogy to similar acylations of acetyl derivatives of aniline[5] and 1-aminonaphthalene,[6] succinoylation of naphthostyril afforded III, with the acyl group *para* to the amido nitrogen. Attempts at Clemmensen reduction of III or its methyl ester (IV) were unsuccessful. However, Wolff–Kishner reduction under the conditions of Huang-Minlon[7] gave γ-(5-naphthostyril)butyric acid (V). Cyclization of V with polyphosphoric acid[8] proceeded smoothly to yield 1-keto-9-amino-1,2,3,4-tetrahydrophenanthrene-8-carboxylic lactam (VIII). Huang-Minlon reduction of VIII gave VII. Lactam VII was hydrolyzed with NaOH in refluxing aqueous dioxane, and the liberated amino acid was directly converted, *via* a Sandmeyer reaction,[9,10] to 9-nitro-1,2,3,4-tetrahydrophenanthrene-8-carboxylic acid (XI) in 37% yield. The Sandmeyer reaction was markedly pH dependent, and a satisfactory yield was obtained only at about pH 6.5. Under more strongly acidic conditions the yield of desired product decreased, and the principal isolable product was 9-hydroxy-1,2,3,4-tetrahydrophenanthrene-8-carboxylic acid lactone (VI). An alternative projected route to XI was VIII → XIII → XI. However, the poor yield in the Sandmeyer-type conversion of VIII to 1-keto-9-nitro-1,2,3,4-tetrahydrophenanthrene-8-carboxylic acid (XIII) made this approach less practical.

SCHEME I



The structure of XI was proven by decarboxylation to 9-nitro-1,2,3,4-tetrahydrophenanthrene (XII), and this was characterized by conversion to the known 9-amino-1,2,3,4-tetrahydrophenanthrene (IX)[11] and 9-acetylamino-1,2,3,4-tetrahydrophenanthrene (X).[11]

Compounds VII and XI were evaluated for tumor-inhibitory activity against Adenocarcinoma 755 in mice and against human carcinoma of the nasopharynx carried in cell culture (KB).[12] No significant inhibitory

(1) (a) Part XXIV: S. M. Kupchan, T.-H. Yang, G. S. Vasilikiotis, M. H. Barnes, and M. L. King, *J. Am. Chem. Soc.*, **89**, 3075 (1967). (b) This investigation was supported by research grants from the National Cancer Institute (CA-04500) and the American Cancer Society (T-275).

(2) American Foundation for Pharmaceutical Education Fellow, 1963–1965.

(3) S. M. Kupchan and R. W. Doskotch, *J. Med. Pharm. Chem.*, **5**, 657 (1962).

(4) S. M. Kupchan and H. C. Wormser, *J. Org. Chem.*, **30**, 3792 (1965).

(5) J. P. English, R. C. Clapp, Q. P. Cole, and J. Krapcho, *J. Am. Chem. Soc.*, **67**, 2263 (1945).

(6) N. J. Leonard and A. M. Hyson, *ibid.*, **71**, 1392 (1949).

(7) Huang-Minlon, *ibid.*, **68**, 2487 (1946).

(8) F. Uhlig and H. R. Snyder, *Advan. Org. Chem.*, **1**, 35 (1960).

(9) H. Goldstein and P. Francey, *Helv. Chim. Acta*, **15**, 1362 (1932).

(10) E. R. Ward, C. D. Johnson, and J. G. Hawkins, *J. Chem. Soc.*, 894 (1960).

(11) W. E. Bachmann and M. W. Cronyn, *J. Org. Chem.*, **8**, 456 (1943).

(12) Cytotoxicity and *in vivo* inhibitory activity were assayed under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health. The procedures were those described in *Cancer Chemotherapy Rept.*, **25**, 1 (1962).