molecular positions in the partially deuterated griseofulvin (Table II), which results from the incorporation of deuterium during biosynthesis at the various proton sites in the molecule. The amount of deuterium incorporated varies with the particular proton site. From Table II it can be seen that replacement of hydrogen by deuterium is maximal at the aromatic and vinyl positions. These molecular sites apparently afford little opportunity for biosynthetic incorporation of proton from the protio substrate, and a maximum of solvent D_2O equilibration occurs at these loci. Decreasing amounts of isotopic substitution occur at the OCH_3, C—CH_3, and CH_2—CH positions, indicating that the protio sugars have a more extensive role in the biosynthetic mechanisms leading to the formation of these moieties. In addition, these sites probably have a decreased capability for equilibration with the solvent. The isotope fractionation factor K_H/K_D calculated as the average of all molecular positions in partially deuterated griseofulvin was found to be 0.36. This indicates a ratio of approximately one hydrogen atom for every three deuterium atoms in the molecule, and an average deuterium isotope replacement of about 75%.

CONCLUSIONS

Griseofulvin extracted from the mycelium and culture filtrates of *P. janczewskii* was purified by preparative thin-layer chromatography and characterized by visible, infrared and nuclear magnetic resonance spectra. Fully deuterated griseofulvin, the first fully deuterated antibiotic so far prepared, was obtained from organisms grown on a fully deuterated medium by direct fermentation. A yield of over 5 mg. was obtained from 500 ml. of culture medium. When a protio medium used for vegetative growth was replaced with a fully deuterated medium, fully deuterated griseofulvin was isolated. Approximately the same amount of antibiotic was obtained from 500 ml. of replacement medium. By this procedure the 50–60 days necessary for maximum deuterio griseofulvin production by direct fermentation could be reduced by about one-half. When the replacement medium was only partially deuterated (protio sugars and pure D_2O), an antibiotic was isolated which was deuterated in part. A yield of 4–6 mg. was obtained from 500 ml. of partially deuterated replacement medium.

Subsequent studies are concerned with the determination of the effect of deuterium on the antibiotic activity. If detoxification of griseofulvin requires rupture of carbon-to-hydrogen bonds it is expected that the deuterio analog should exhibit an enhanced activity since carbon-to-deuterium bonds are generally more stable. A statistical analysis of antifungal activity of deuterio griseofulvin will be the subject of the next report.

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Keyphrases

Penicillium janczewskii II—cultures
Deuterium oxide—*P. janczewskii II* cultures
Griseofulvin, fully deuterated—isolation
TLC—separation
Silica gel chromatohile—separation
NMR spectrometry—identity
IR spectrophotometry—structure
UV spectrophotometry—structure

Synthesis of 2-(*N*,*N*-Dialkylaminomethyl)-7-phenylthioindoles

By ROBERT L. DUNCAN, JR.* and JOHN ANDRAKO†

A series of 2-(*N*,*N*-dialkylaminomethyl)-7-phenylthioindoles was prepared. The Japp-Klingemann reaction followed by Fisher cyclization with polyphosphoric acid gave 7-phenylthioindole-2-carboxylic acid. The lithium aluminum hydride reduction of amides derived from this acid provided the corresponding aminomethyl indoles. Infrared and NMR data are reported.

As part of a program directed at the synthesis and pharmacological evaluation of dialkylaminoalkyl-7-substituted indoles, the authors wish to report the synthesis of a series of 2-(*N*,*N*-dialkylaminomethyl)-7-phenylthioindoles (VI). Relatively few 7-substituted indoles and indole derivatives have been reported and there is an almost total lack of pharmacological data for compounds of this type. Hiremath and Siddappa (1) reported the synthesis of 5-methoxy-7-

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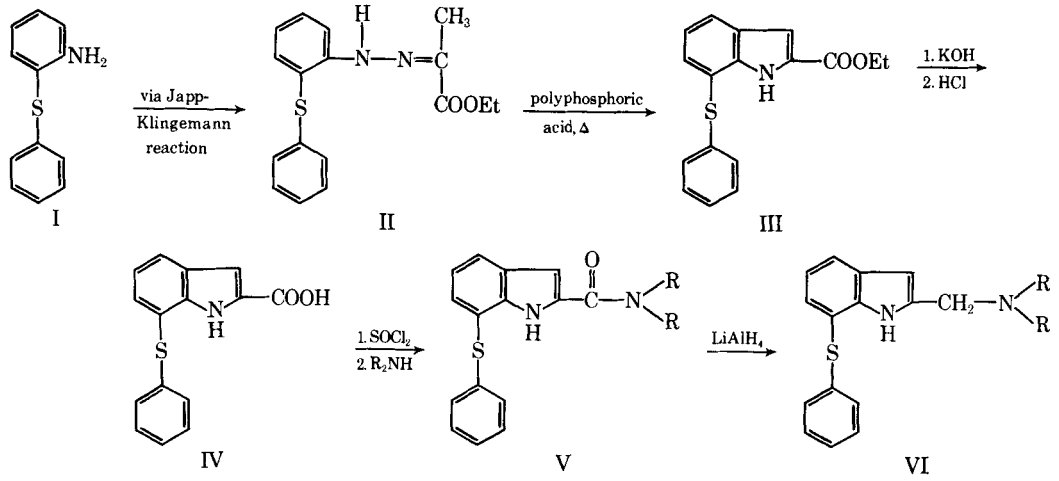
nitroindole, 5-methyl-7-nitroindole, and the corresponding aminoindoles as possible antiserotonin agents, but did not report any biological data for these compounds. The syntheses of a number of 2-dialkylaminomethylindole derivatives have been reported by Kornfeld (2), Yoneda, and co-workers (3) and others (4–6).

The 2-dialkylamino-7-phenylthioindoles are of particular interest since they may be related structurally to "open" phenothiazines.

DISCUSSION

The synthesis of the reported compounds was accomplished by the sequence of reactions outlined in Scheme I.



Scheme I

The *o*-(phenylthio)aniline (I) was obtained in good yield by reduction of *o*-(phenylthio)nitrobenzene which was prepared by treating sodium thiophenoxide with *o*-chloronitrobenzene. Diazotization of I and reaction of the resulting diazonium compound with ethyl 2-methylacetoacetate under Japp-Klingemann conditions yielded the desired ethyl pyruvate *o*-(phenylthio)-phenylhydrazone in about 50% yield. An attempt to obtain *o*-(phenylthio)phenylhydrazine by reduction of the diazonium compound of I with stannous chloride and hydrochloric acid was made. A solid was obtained which on the basis of its infrared absorption at 2260 cm.$^{-1}$ (N ≡ N) (7) appeared to be a stable tin double salt of diazotized II. No further characterization of the solid was made.

The indole ester, III, was prepared by cyclization of II using polyphosphoric acid. When the temperature of the reaction mixture was raised to 90° an exothermic reaction occurred. At this point the reaction was immediately quenched with ice water. If the reaction mixture was maintained at 90° for longer periods of time, extensive tar formation occurred, the yields of indole ester were lower, and it was more difficult to purify the small amounts of product which were isolated. Attempts to bring about the cyclization of II using a saturated ethanolic solution of hydrogen chloride or ethanolic zinc chloride were unsuccessful.

The 7-phenylthioindole-2-carboxylic acid, was obtained by hydrolysis of the ester (III) with potassium hydroxide followed by acidification with hydrochloric acid. The indole acid was converted to the acid chloride using thionyl chloride and was used without prior isolation to prepare the carboxamides, V*a*, *b*, *c*, *d*, described in Table I. Conversions of indole acids to indole acid chlorides have been reported (2, 8, 9).

The carboxamides were reduced with lithium aluminum hydride to the corresponding 2-(*N*,*N*-dialkylaminomethyl)-7-phenylthioindoles, VI*a*, *b*, *c*, *d*, indicated in Table II.

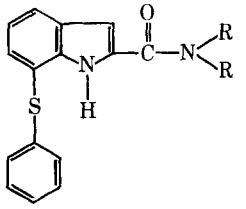
The infrared and NMR spectra are in agreement with the assigned structures at each step indicated in Scheme I.

EXPERIMENTAL[1]

o-(Phenylthio)nitrobenzene—To a sodium ethoxide solution prepared by adding slowly 20.7 Gm. (0.9 mole) of sodium metal to 300 ml. of absolute ethanol there was added in portions over a period of 20 min., 110 Gm. (1.0 mole) of benzenethiol followed by 157.6 Gm. (1.0 mole) of *o*-chloronitrobenzene.

[1] Melting points were obtained with a Thomas-Hoover capillary melting point apparatus and are uncorrected. All analyses were performed at the Medical College of Virginia unless otherwise indicated. Infrared spectra were obtained with a Beckman IR-8 spectrophotometer. NMR data were obtained with a Varian Associates model A60 NMR spectrometer through the courtesy of the A. H. Robins Co., Richmond, Va.

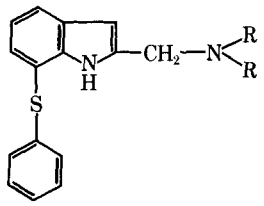
TABLE I—7-PHENYLTHIOINDOLE-2-CARBOXAMIDES



Compound	—N(R)R	M.p. °C.	Yield, %	Formula	Anal. Calcd.	Anal. Found
V *a*	—N(CH_3)$_2$	191–193[a]	82	$C_{17}H_{16}N_2OS$	C, 68.89 H, 5.44 N, 9.45	C, 68.56 H, 5.54 N, 8.9
V *b*	—N(C_2H_5)$_2$	168–169[a]	54	$C_{19}H_{20}N_2OS$	C, 70.34 H, 6.21 N, 8.64 S, 9.88	C, 69.84[b] H, 6.38[b] N, 8.52[b] S, 9.84[b]
V *c*	—N⬡	124–125.5[a]	64	$C_{20}H_{20}N_2OS$	C, 71.40 H, 5.99 N, 8.33 S, 9.53	C, 71.38[b] H, 5.98[b] N, 8.61[b] S, 9.46[b]
V *d*	—N⬡O	86–88[a]	59	$C_{19}H_{18}N_2O_2S$	C, 67.43 H, 5.36	C, 67.12 H, 5.61

[a] Recrystallized from methanol. [b] Analysis by Microtech Labs., Skokie, Ill.

TABLE II—2-(*N*,*N*-DIALKYLAMINOMETHYL)-7-PHENYLTHIOINDOLES



Compound	—N(R)R	M.p., °C.	Yield, %	Formula[b]	Anal. Calcd.	Anal. Found
VI*a*	—N(CH_3)$_2$	164–166[a]	74	$C_{17}H_{19}ClN_2S$	C, 64.03 H, 6.01 N, 8.70	C, 63.56 H, 6.18 N, 8.5
VI*b*	—N(C_2H_5)$_2$	120–122[a]	86	$C_{19}H_{23}ClN_2S$	C, 65.78 H, 6.68 N, 8.08	C, 65.49 H, 6.91 N, 7.9
VI*c*	—N⬡	207–208.5[a]	81	$C_{20}H_{33}Cl_3N_2S$	C, 66.92 H, 6.46 N, 7.81	C, 66.95 H, 6.90 N, 7.68
VI*d*	—N⬡O	187–188[a]	93	$C_{19}H_{21}ClN_2OS$	C, 63.23 H, 5.87 N, 7.76	C, 63.54 H, 6.33 N, 7.25

[a] Recrystallized from 2-butanone. [b] Hydrochlorides.

The mixture was allowed to reflux for 3.5 hr. After the addition of 500 ml. of water, the mixture was extracted with ether. The combined ethereal extracts were dried (anhydrous sodium sulfate) and concentrated. The solid residue thus obtained weighed 154.4 Gm. (74.2%) and after recrystalliza-

tion from ligroin melted at 77–79° [lit. 77° (10); 78–80° (11)].

o-(Phenylthio)aniline (I)—A solution of 43.1 Gm. (0.23 mole) of stannous chloride in 106 ml. of concentrated hydrochloric acid was added slowly to a stirred solution of 11.6 Gm. (0.05 mole) of *o*-(phenylthio)nitrobenzene. The solution was heated on a steam bath until the yellow color disappeared (2–3 hr.). The solution was cooled, carefully made basic with sodium hydroxide, and extracted with ether. The ethereal extracts were combined, dried (anhydrous sodium sulfate), and concentrated. The residual oil, upon standing in the freezer, crystallized and 7.1 Gm. (70%) of pale yellow crystals was obtained which melted at 34–36° [lit. 33° (11)]. The acetyl derivative melted at 84–86° [lit. 86° (10)].

Ethyl Pyruvate o-(Phenylthio)phenylhydrazone (II)—A solution of 60.4 Gm. (0.3 mole) of *o*-(phenylthio)aniline in 270 ml. of 6 *N* hydrochloric acid contained in a three-necked flask was cooled in an ice-salt bath and stirred. While maintaining the temperature of the reaction mixture at 0–5°, 22.1 Gm. (0.32 mole) of sodium nitrite in 60 ml. of water was added dropwise. The solution of the diazonium salt was maintained at 0° until it was used later. A solution of 44.6 Gm. (0.31 mole) of ethyl 2-methylacetoacetate in 300 ml. of ethanol was stirred and cooled to 0°, and 108 ml. of a 50% solution of potassium hydroxide was added with vigorous stirring. About 400 ml. of an ice–water mixture was added to the reaction mixture, and the previously prepared cold solution of the diazonium salt was introduced immediately. The red mixture was stirred for 16 hr. during which time it was allowed to come to room temperature. The aqueous reaction mixture was extracted with ether. The ethereal extracts were combined, and the ether was evaporated leaving a dark red oil which resisted all attempts at crystallization. The crude oil was distilled with care and the fraction boiling at 160–165°/0.05 mm. was collected, yielding 46.0 Gm. (49%) of the hydrazone ester. The infrared spectrum in chloroform showed $\nu_{max.}$ 3300 cm.$^{-1}$, 3220 cm.$^{-1}$, 1700 cm.$^{-1}$.

Anal.—Calcd. for $C_{17}H_{18}N_2O_2S$: C, 64.94; H, 5.77; N, 8.91. Found: C, 64.63; H, 5.73; N, 8.83.

Ethyl 7-(Phenylthio)indole-2-carboxylate (III)—A mixture of 45 Gm. (0.15 mole) of II and 120 Gm. of polyphosphoric acid was stirred in a 500-ml. conical flask equipped with a thermometer. The mixture was carefully heated to 90° whereupon an exothermic reaction occurred. At this point about 100 ml. of ice water was added immediately to the reaction mixture. When decomposition of the excess polyphosphoric acid was complete, the semi-solid ester was collected by filtration. The residue was dissolved in 100 ml. of ether, and the solution was dried and concentrated. A gummy residue which weighed 30.0 Gm. (71%) was obtained. Upon standing, crystals formed in the residue. After recrystallization from ethanol, the yellow crystals melted at 133–135°. The infrared spectrum (KBr disk) showed $\nu_{max.}$ 3350 cm.$^{-1}$, 1675 cm^{-1}.

Anal.—Calcd. for $C_{17}H_{15}NO_2S$: C, 68.66; H, 5.09; N, 4.71. Found: C, 68.71; H, 5.67; N, 4.25.

7-Phenylthioindole-2-carboxylic Acid (IV)—To a solution of 101 Gm. (0.34 mole) of III in 200 ml. of absolute ethanol was added an excess of aqueous potassium hydroxide solution prepared by dissolving 50 Gm. of potassium hydroxide in 50 ml. of water. The solution was refluxed for 5 hr. After standing 16 hr. the solution was diluted with 500 ml. of water and extracted with chloroform. The aqueous layer was made acidic with 5% hydrochloric acid solution, and a red oil, which crystallized when scratched with a glass rod, separated from solution. The solid was filtered with suction, washed with water, and air dried. The crude solid (75 Gm.) was dissolved in 100 ml. of a 5% sodium bicarbonate solution and the resulting solution was extracted with both chloroform and ether. The aqueous solution was treated with charcoal and warmed to remove any remaining organic solvent. The mixture was filtered and the filtrate made acidic with a 5% hydrochloric acid solution. The acid which precipitated was collected by filtration and dissolved in methanol. When water was added, a pale yellow solid separated which was removed by filtration and air dried. Decolorization with charcoal and recrystallization from methanol–water gave 60 Gm. (91%) of the crude acid which melted at 150–160°. Recrystallization from benzene gave a crystalline product which melted at 158.5–160.5°. The infrared spectrum (KBr disk) showed $\nu_{max.}$ 3410 cm.$^{-1}$, 1660 cm.$^{-1}$. The NMR spectrum (DMSO) showed only an aromatic multiplet, extending from 6.9–7.9 δ, an acid singlet at 12.3 δ, and a broad NH area in the region of 8.5 δ. The integration of 1:1:10 for the acid, NH, and aromatic areas, respectively, agreed with the assigned structure.

Anal.[2]—Calcd. for $C_{14}H_{11}NO_2S$: C, 66.89; H, 4.12; N, 5.20; S, 11.90. Found: C, 66.67; H, 4.16; N, 5.05; S, 11.86.

(N,N-Dimethyl) - 7 - phenylthioindole - 2 - carboxamide (Va)—A solution of 5.0 Gm. (0.019 mole) of IV in 50 ml. of thionyl chloride was stirred for 2 hr. at room temperature. The thionyl chloride was removed under vacuum, and the residue which remained was dissolved in 30 ml. of tetrahydrofuran. A solution of 1.6 Gm. (0.038 mole) of dimethylamine in tetrahydrofuran was added dropwise from a dropping funnel to the solution of the acid chloride. After 15 min. of stirring, the mixture was filtered and the filtrate was evaporated to dryness. The residue which remained was dissolved in 50 ml. of chloroform, and the chloroform solution was extracted successively with 15-ml. portions of 5% hydrochloric acid solution, 5% sodium hydroxide solution, and water. The chloroform layer was evaporated to dryness and the 4.5 Gm. (82%) of crude amide which remained was dissolved in hot methanol, decolorized with charcoal, and filtered. The white crystalline amide which separated melted at 191–193°. The infrared spectrum ($CHCl_3$) showed $\nu_{max.}$ 3440 cm.$^{-1}$, 1600 cm.$^{-1}$. The NMR spectrum ($CDCl_3$) showed a singlet at 3.07 δ (CH_3), a multiplet at 7.25 δ (aromatic), and a broad NH absorption band far downfield. Other data are in Table I.

(N,N - Diethyl) - 7 - phenylthioindole - 2 - carboxamide (Vb)—The procedure described for *Va* was followed. The infrared spectrum ($CHCl_3$) showed $\nu_{max.}$ 3440 cm.$^{-1}$, 1600 cm.$^{-1}$. The NMR spectrum ($CDCl_3$) showed a triplet at 1.1 δ (CH_3), a quartet at 3.5 δ (CH_2), and a multiplet at 7.25 δ (aromatic). Other data are in Table I.

1-(7-Phenylthio-2-indolylcarbonyl)piperidine (Vc)—The procedure described for *Va* was followed.

[2] Analyses by Microtech Laboratories, Skokie, Ill.

The infrared spectrum (KBr disk) showed $\nu_{max.}$ 3440 cm.$^{-1}$, 1600 cm.$^{-1}$. Other data are in Table I.

4 - (7 - Phenylthio - 2 - indolylcarbonyl)morpholine (Vd)—The procedure described for V*a* was followed. The infrared spectrum ($CHCl_3$) showed $\nu_{max.}$ 3440 cm.$^{-1}$, 1600 cm.$^{-1}$. Other data are in Table I.

2 - (N,N - Dimethylaminomethyl) - 7 - phenyl thioindole (VIa)—Lithium aluminum hydride, 540 mg. (0.015 mole) was added in portions to 75 ml. of tetrahydrofuran. The mixture was stirred for 15 min. A solution of 2.0 Gm. (0.007 mole) of (*N,N*-dimethyl)-7-phenylthioindole-2-carboxamide in 25 ml. of tetrahydrofuran was slowly added and the mixture was stirred for an additional 30 min. Water was added dropwise with caution until no visible reaction could be detected, and the mixture was stirred for an additional 15 min. After filtration and evaporation of the solvent, the oily residue which remained was dissolved in ether. The ether solution was extracted with 30 ml. of 5% hydrochloric acid solution and the amine hydrochloride separated as an oil. The hydrochloride and the acidic aqueous layer were combined and made basic with 5% sodium hydroxide solution. After extracting with ether, the ethereal extracts were dried. Addition of an excess of ethereal hydrogen chloride precipitated the white hydrochloride salt as a solid. Recrystallization from 2-butanone gave 1.6 Gm. (74%) of crystalline hydrochloride melting at 164–166°. The infrared spectrum of the free amine ($CHCl_3$) showed $\nu_{max.}$ 3440 cm.$^{-1}$. The NMR spectrum ($CDCl_3$) of the hydrochloride showed a singlet at 2.75 δ (CH_3), a singlet at 4.45 δ (CH_2), a singlet at 6.6 δ (3 position proton), a multiplet at 7.2 δ (aromatic), and the NH and HCl proton absorption bands far downfield. Other data are in Table II.

2-(N,N-Diethylaminomethyl)-7-phenylthioindole (VIb)—The procedure described for VI*a* was followed. The infrared spectrum of the free amine ($CHCl_3$) showed $\nu_{max.}$ 3450 cm.$^{-1}$. The NMR spectrum of the free amine ($CDCl_3$) showed a triplet at 0.9 δ (CH_3), a quartet at 2.45 δ (CH_2), a singlet at 3.6 δ (CH_2), a singlet at 6.3 δ (3 position proton), a multiplet at 7.2 δ (aromatic), and an NH absorption band far downfield. Other data are in Table II.

7-Phenylthio-2-piperidinomethylindole (VIc)—The procedure described for VI*a* was followed. The infrared spectrum of the hydrochloride ($CHCl_3$) showed a $\nu_{max.}$ 3225 cm.$^{-1}$ and a broad NH absorption band in the region 2630 cm.$^{-1}$ and 2120 cm.$^{-1}$. The NMR spectrum of the hydrochloride ($CDCl_3$) showed multiplets at 1.8, 2.8, and 3.2 δ (piperidinomethylenes), a doublet at 4.3 δ (CH_2), a singlet at 6.55 δ (3 position proton), a multiplet at 7.2 δ (aromatic), and the NH and HCl proton absorption bands far downfield. Other data are in Table II.

2-Morpholinomethyl-7-phenylthioindole (VId)—The procedure described for VI*a* was followed. The infrared spectrum of the hydrochloride ($CHCl_3$) showed $\nu_{max.}$ 3225 cm.$^{-1}$ and a broad NH absorption band in the region 2630 cm.$^{-1}$ and 2120 cm.$^{-1}$. The NMR spectrum of the hydrochloride ($CDCl_3$) showed multiplets at 3.15 and 3.9 δ (morpholino methylenes), a singlet at 4.4 δ (CH_2), a singlet at 6.6 δ (3 position proton), a multiplet at 7.2 δ (aromatic), and the NH and HCl proton absorption bands far downfield.

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Keyphrases

2-(*N,N*-Dialkylaminomethyl)-7-phenylthioindoles—synthesis
IR spectrophotometry—structure
NMR spectrometry—identity