crinology, 88, 633 (1971).
(9) R. Walter, H. Shlank, J. D. Glass, I. L. Schwartz, and T. D. Kerenyi, *Science*, 173, 827 (1971).
(10) R. Walter, *Peptides, Proc. Eur. Symp., 12th*, 363 (1973).
(11) L. Fruhaufová, E. Suska-Brzezińska, T. Barth, and I. Rychlík, *Collect. Czech. Chem. Commun.*, 38, 2793 (1973).
(12) E. Schillinger, O. Loge, E. Schröder, E. Klieger, and K. Lübke, *Eur. J. Biochem.*, 27, 473 (1972).
(13) R. B. Merrifield, *J. Amer. Chem. Soc.*, 85, 2149 (1963); *Advan. Enzymol.*, 32, 221 (1969).
(14) J. C. Sheehan and G. P. Hess, *J. Amer. Chem. Soc.*, 77, 1067 (1955).
(15) W. König and R. Geiger, *Chem. Ber.*, 103, 788 (1970).
(16) E. Sandrin and R. A. Boissonnas, *Helv. Chim. Acta*, 46, 1637 (1963).
(17) E. Schröder and E. Klieger, *Justus Liebigs Ann. Chem.*, 673, 208 (1964).
(18) H. Zahn, W. Danho, and B. Gutte, *Z. Naturforsch. B*, 21, 763 (1966).
(19) M. Manning, *J. Amer. Chem. Soc.*, 90, 1348 (1968).
(20) J. M. Stewart and J. D. Young, "Solid Phase Peptide Synthesis," W. H. Freeman, San Francisco, Calif., 1969.
(21) R. A. Sifferd and V. du Vigneaud, *J. Biol. Chem.*, 108, 753 (1935).
(22) V. du Vigneaud, C. Ressler, J. M. Swan, C. W. Roberts, P. G. Katsoyannis, and S. Gordon, *J. Amer. Chem. Soc.*, 75, 4879 (1953).
(23) D. Yamashiro, *Nature (London)*, 201, 76 (1964).
(24) L. Y. Sklyarov and I. V. Shaskova, *Zh. Obshch. Khim.*, 39, 2779 (1969).
(25) J. Meienhofer and A. Trzeciak, *Proc. Nat. Acad. Sci. U. S.*, 68, 1006 (1971).
(26) J. D. Glass, I. L. Schwartz, and R. Walter, *J. Amer. Chem. Soc.*, 94, 6209 (1972).
(27) J. D. Glass, R. Walter, and I. L. Schwartz, *Peptides, Proc. Eur. Symp., 12th*, 135 (1973).
(28) J. D. Glass, A. Talansky, Z. Grzonka, I. L. Schwartz, and R. Walter, *J. Amer. Chem. Soc.*, in press.
(29) H. Aoyagi, M. Kondo, and N. Izumiya, *Bull. Chem. Soc. Jap.*, 41, 2772 (1968).
(30) H. Takashima, W. Fraefel, and V. du Vigneaud, *J. Amer. Chem. Soc.*, 91, 6182 (1969).
(31) R. Walter, J. D. Glass, B. M. Dubois, M. Koida, and I. L. Schwartz, *Peptides: Chem. Biochem., Proc. Amer. Peptide Symp., 2nd*, 327 (1972).
(32) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.*, 30, 1190 (1958).
(33) P. Lefrancier and E. Bricas, *Bull. Soc. Chim. Biol.*, 49, 1257 (1967).
(34) E. Kaiser, R. L. Colescott, C. D. Bessinger, and P. I. Cook, *Anal. Biochem.*, 34, 595 (1970).
(35) G. Ellman, *Arch. Biochem. Biophys.*, 82, 70 (1959).
(36) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, 193, 265 (1951).
(37) R. Walter and R. T. Havran, *Experientia*, 27, 645 (1971).
(38) O. Warburg and W. Christian, *Biochem. Z.*, 310, 384 (1941).
(39) J. D. Glass, B. M. Dubois, I. L. Schwartz, and R. Walter, *Endocrinology*, 87, 730 (1970).
(40) S. Hase and R. Walter, *Int. J. Protein Res.*, 5, 283 (1973).
(41) P. Holton, *Brit. J. Pharmacol.*, 3, 328 (1948).
(42) R. A. Munsick, *Endocrinology*, 66, 451 (1960).
(43) R. A. Munsick, W. H. Sawyer, and H. B. Van Dyke, *Endocrinology*, 66, 860 (1960).
(44) J. M. Coon, *Arch. Int. Pharmacodyn.*, 62, 79 (1939).
(45) W. A. Jeffers, J. J. Livezey, and J. H. Austin, *Proc. Soc. Exp. Biol. Med.*, 50, 184 (1942).
(46) "The Pharmacopeia of the United States," 17th revision, Mack Publishing Co., Easton, Pa., 1965, p 749.

Effect of Lipophilic Substituents on Some Biological Properties of Indoles

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Simple indole derivatives were screened for antimicrobial activity. Although the majority of these indoles failed to inhibit the growth of any of the test microbes, some compounds were effective inhibitors at relatively low concentrations. Indoleacetic acids and carboxylic acids were the least active, aminomethyl derivatives were moderately active, and 5-, 6-, and 7-haloindoles were the most active of the simple indoles. Diarylmethyl substituents further enhanced the potency of the simple indoles including the active halo derivatives and also converted some inactive indoles into effective growth inhibitors. Toxicity, behavioral effects, antiviral activity, antiinflammatory properties, antispasmotic activity, and general endocrine properties of the (diarylmethyl)indoles are described.

Simple indole derivatives demonstrate numerous biological effects. Indole, 3-methylindole, 3-indoleacetic acid, and tryptophan delay the spread of tuberculosis in guinea pigs and 1-methylindole-2,3-dione 3-thiosemicarbazone is useful in the prophylaxis of smallpox.[1] Indolylalkylamines induce responses in the central nervous system and in peripheral organs and tissues of higher animals.[2-9] Carboxylic acid derivatives of indole are effective antiinflammatory agents.[10] A study of the antimicrobial activity of indoles, therefore, would not be complete nor practical without a knowledge of the compounds effects upon the host animal.

In this investigation, the antimicrobial structure-activity relations were correlated for 400 simple indole derivatives. The correlations served as references for establishing the effect of lipophilic groups on this activity. (Diarylmethyl)indoles were prepared by carbonium ion alkylations of selected indoles. The modified indoles were then screened for antibacterial, antifungal, antiprotozoal, and antiviral activities. Finally, they were studied for biological reactions that might appear as clinical manifestations in a host animal. These included toxic effects, behavioral changes, antiinflammatory activity in animals, antagonist reactions on isolated smooth muscle, and general endocrine properties.

The antimicrobial screening procedure employed by W. Wick and associates of these laboratories was essentially that described by Johnson.[11] Each of the 51 microbes (Table I) was inhibited by at least one of the indole compounds at a concentration of 200 μg/ml. Indole and 46% of the 400 variously substituted indoles that were tested failed to inhibit any of the organisms. A partial, but representative list of the inactive compounds is given in Table II.† The remaining 54% inhibited at least one species, either a bacterial or fungal organism. Of all the compounds tested, 36% inhibited at least two species and finally 8% of the compounds inhibited at least 15 species.

In order to establish substituent-activity relationships, the indoles were classified into groups having similar substituents. Furthermore, all active compounds were reviewed so that each important substituent-activity effect,

† See paragraph at end of paper regarding supplementary material.

regardless of the class of compound, would be recognized. Of the simple indole derivatives, 35% were carboxylic acids, amides, esters, and hydrazides; 18% were amines; and 12% were halogen derivatives. Other less prevalent indole types were substituted with aldehyde, alkoxy, alkyl, aryl, benzoyl, benzyl, benzyloxy, cyano, hydroxy, keto, nitro, trifluoromethyl, and methylene groups.

The most active compounds are listed in Table III. Ethyl 3-indolylacrylate, 5-bromo-3-(2-cyanovinyl)indole, and 3-(2-nitrovinyl)indole (1-3, Table III) are distinctive in that their antimicrobial activity is probably due to the active double bond which reacts with sulfhydryl enzymes. These methylene derivatives have a wide spectrum of activity and are effective at concentrations of 10-100 μg/ml. Miscellaneous compounds, 5-methylindole (4), 6-(trifluoromethyl)indole (5), 5-(benzyloxy)indole (6), 5-, 6-, and 7-nitroindoles (7-10), and 5-bromo-3-indolecarboxaldehyde (11), were effective at 100 μg/ml.

Indolecarboxylic, acetic, propionic, and butyric acids and their amides, esters, and hydrazides had a low incidence of activity, and the most active examples were effective at relatively high concentrations (12, 13, Table III).

The amino derivatives had a higher incidence of activity; *i.e.*, 59% inhibited one or more and 9% inhibited 15 or more organisms. However, the amines had erratic activity. Compounds 14-20 (Table III) were effective in the range of 10-50 μg/ml. Other amines, not identified here but similar in structure, were active only at much higher concentrations or not active at all. This difference in antimicrobial activity is not apparent from dissimilarities in gross structure. Haloindoles were the most active compounds of the simple indoles. Of all the haloindoles, selected at random, 80% inhibited the growth of at least one test organism and 32% prevented the growth of 15 or more species. The 3-, 4-, 5-, 6-, and 7-monochloroindoles (21-27, Table III) were effective at concentrations of 10-100 μg/ml. Bromine and chlorine atoms in the 5 or 6 positions of indole (22, 25, and 28, Table III) had the greatest potentiating effect.

The compound 5-bromoindole (28, Table III), at a concentration of 10 μg/ml, inhibited six of the fungal species and was considered to be the most effective antifungal agent of the simple indoles.

Indole carboxylic and alkylcarboxylic acids were the least effective. With the exception of β-[3-indolyl]propionic acid (12, Table III) other examples, compounds 29 and 30, had no antifungal activity and limited antibacterial activity. Indoles substituted at either the 2 or 3 position with alkyl, hydroxyalkyl, amide, ester, aldehyde, and keto groups (see compounds 32-39, Table III) had an incidence of activity lower than that observed for amino- and haloindoles. On the other hand, alkyl, trifluoromethyl, benzyloxy, and hydroxy groups, in the 4, 5, 6, or 7-positions of indole, appeared to enhance antimicrobial activity.

The (diarylmethyl)indole derivatives had the highest incidence of activity against 1-6 bacteria but the activity dropped precipitously for more than six bacterial species. Antifungal activity for this group of compounds was about the lowest of all substitutent classes. Simple indole derivatives with mixed antifungal and antibacterial activities were therefore converted, principally, to antibacterial compounds by introducing an appropriate diarylmethyl substituent. The activity of 5-bromoindole (28, Table III) was increased 2-25 times by adding the diphenylmethyl group to the 3 position. This 5-bromo-3-(diphenylmethyl)-indole (40, Table IV) was the most effective antibacterial indole derivative of the series and was effective at 0.78-10 μg/ml against both gram-negative and gram-positive bacteria (Table IX). This activity is in the range of clinically important antibiotics which are effective in the following concentrations (μg/ml): penicillin potassium G, 0.01-1000, penicillin potassium V, 0.02-1000; erythromycin glucoheptonate, 0.1-250; streptomycin sulfate, 10-100; vancomycin, 0.78-1000; tylosin, 0.2-1000; tetracycline hydrochloride, 0.16-12.5; chloramphenicol, 0.16-1000; novobiocin sodium, 0.16-1000; and capreolimycin, 62-1000.

Table I. Test Organisms

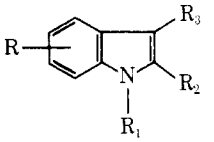
Bacteria	Fungi
(a) *S. aureus*	I, *T. mentagrophytes*
(b) *B. subtilis*	II, *T. interdigitale*
(c) *M. avium*	III, *T. rubrum*
(d) *M. tuberculosis* 607	IV, *A. solani*
	V, *A. niger*
(e) *S. faecalis*	VI, *B. cinerea*
(f) *L. casei*	VII, *C. ulmi*
(g) *L. citrovorium*	VIII, *C. resinae*
(h) *A. tumefaciens*	IX, *C. pisi*
(i) *E. amylovora*	X, *C. lagenarium*
(j) *E. caratovora*	XI, *C. gossypii*
(k) *Ps. solanacearum*	XII, *C. phomoides*
(l) *Ps. species* no. 2 and no. 5	XIII, *F. oxysporium lycopersici*
(m) *X. phaseoli*	XIV, *F. moniliforme*
	XV, *C. albicans*
(n) *X. pruni*	XVII, *M. fructicola*
(o) *E. coli* no. 1 and no. 2	XVIII, *U. avenae*
	XIX, *A. imperfecta*
(p) *K. aerobacter* no. 14 and no. 15	XX, *E. fagacearum*
	XXI, *G. cingulata*
(q) *K. pneumoniae*	XXII, *H. sativum*
(r) *P. species* no. 1 and no. 2	XXIII, *P. expansum*
	XXIV, *P. species*
(s) *V. metschnilorii*	XXV, *S. fructicola*
(t) *V. cholera*	XXVI, *V. albo-atrum*
(u) *B. bronchisiptica*	XXVII, *M. ramannianus*
(v) *C. sepodonicum*	XXVIII, *S. divaricata*
(w) *C. michiganense*	

The 3-diarylmethyl derivatives of indolecarboxylic acids (41-52, Tables IV-VII) inhibited the growth of bacteria at 3.12-12.5 μg/ml (Table IX) while the parent compounds were inactive at 100-200 μg/ml. Substituents on the benzene rings of the diarylmethyl groups had a great influence upon antimicrobial activity. Alkoxy substituents negated the potentiating action of the large group, at least in the examples of compounds 53-58 (Table IX). Halogen substituents increased the potentiating action (46-48, Table V).

Indole did not inhibit the growth of the test microbes, but 3-(diphenylmethyl)indole (59, Table VI) had good activity against *Mycobacterium avium*, and 3-xanthen-9-ylindole (60, Table VI) at 10 μg/ml inhibited two bacteria and two fungi. 3-(Diphenylmethyl)-1-methylindole (61, Table VI) was not an inhibitor at 200 μg/ml. Larger triphenylmethyl, triphenylethyl, and triphenylpropionyl groups in position 3 of indole (62-64, Table VI) did not contribute to activity (Table IX). It is also apparent that the di- and triarylmethyl groups placed on the 2 position of 3-indoleacetic, -propionic, and -butyric acids (58, 65-69, Tables VII-VIII) are not appropriate for antimicrobial activity (Table IX). Halo-substituted diarylmethyl groups in this position are expected to have greater probable potentiation.

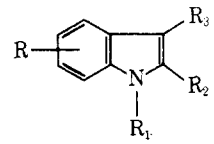
Amine functions on the diarylmethyl group (70, 71, Ta-

Table III. Antimicrobial Activities of Simple Indole Derivatives



Compd no.	R	R_1	R_2	R_3	Compd concn, μg/ml, followed by microorganism designation
1	H	H	H	$CH{=}CHCO_2C_2H_5$[a]	10, IX X XII; 50, cds II–IV VI XVI XX–XXII; 100, a himq VII XIV XXVI; 200, bo V XXIII
2	H	H	H	CH=CHCN	100, a chiklms I IV V VII–IX XIII XVII XXI–XXVIII
3	5-Br	H	H	$CH{=}CHNO_2$	10, c I IV–IX XIII XVII XXI XXII XXVII; 100, abefs XV
4	5-CH_3	H	H	H	100, achlms I IV–VII IX XII XVI XXI–XXIII XXV XXVIII
5	6-F_3C	H	H	H[b]	10, c VI; 100, abdef–iks I IV–IX XIII XXI–XXIII XXV XXVIII
6	5-$C_6H_5CH_2O$	H	H	H	100, ackm IV VI VII IX XV XXII XXV–XXVII
7	5-NO_2	H	H	H	100, ao
8	5-NO_2	H	CH_3	CH_3	100, VI
9	6-NO_2	H	CH_3	CH_3[c]	100, s I IV VI VII IX XVII XXII XXVII
10	7-NO_2	H	H	H	100, bchikms I IV V VII IX XIII XVI XXI–XXVIII
11	5-Br	H	H	CHO	100, hklms IV VI–IX XIII XVII XX XXI XXII XXVI XXVII
12	H	H	H	$(CH_2)CO_2H$	50, km VI XVI; 200, chiw
13	H	H	H	$CH_2CO_2C_2H_5$	50, lm XX XXIII XXVI; 200, cdiqns II III IV VII IX X XII–XV XXI XXII
14	H	H	H	c-NC_5H_4-*p*-CH_2[d]	10, I; 100, cikms IV VI–IX XIII XXII XXV XXVI
15	H	H	H	c-NC_5H_4-*o*-CH_2[d]	10, I; 100, ck IV VI XXII XXV–XXVII
16	H	H	H	$(CH_2)_2NHCH_2C_6H_5$[e]	50, m; 200, uv XVIII XXV
17	5-CH_3O	$C_6H_5CH_2$	CH_3	$(CH_2)_2NH_2$[f]	25, b–d; 50, v II; 200, aikoyu III XI
18	H	H	H	$C_6H_{11}NHCH_2$	100, cks I IV–VII XVII XXI XXII XXVI–XXVIII
19	5-$C_6H_5CH_2O$	H	H	$H_5C_2N(CH_3)(CH_2)_2$	100, cms VI XXVIII
20	H	c-NC_5H_4-*m*-CH_2	H	H[g]	10, k I V VI XXII XXIII; 100, abcsm IV VII–IX XVII XXVI–XXVIII;
21	4-Cl	H	H	H[h]	100, a–ce–ik–moprs I V–IX XII XV XXI–XXIII XXV–XXVIII
22	5-Cl	H	H	H	25, a–ce–g I IV VI XXII XXIII XXV XXVII; 100, hik–prs V VII IX XIII XV XVI XXI XXVII XXVIII
23	H	H	H	Cl[i]	10, bcI; 100, aefgk–m V–IX XIII XXI–XXVIII
24	6-Cl	H	CH_3	H[j]	10, l I IV VI XXII–XXV; 100, bcghik moprs V VII–IX XV XIII XXI XXVI–XXVIII
25	5-Cl	H	CH_3	H	100, adikoqu I VII
26	7-Cl	H	H	H	100, adikoqu I VII
27	6-Cl	H	H	H	100, aiqu I VII; >100, eo
28	5-Br	H	H	H	<10, l I VI VII XXII XXIV XXV; 100, a–cg–ikmoprs IV–VIII IX XIII XVI XXI XXIII XXVII XXVIII
29	5-Cl	H	CO_2H	H	100, c
30	5-$C_6H_5CH_2O$	H	CO_2H	H[k]	100, c
31	H	H	H	F_3CCOCH_2[l]	<10, c I VI XVII; <100, IV XXII
32	H	*n*-C_4H_9	H	H[m]	100, I VI IX XXV XXVII
33	5-CH_3	H	CH_3	C_3H_7[n]	100, a–gs I IV VI XV XXII XXV XXVII
34	H	H	H	H_3CCOCH_2	<10, VI
35	6-CH_3	H	H	CHO[o]	100, chis I VI XIII XXVI

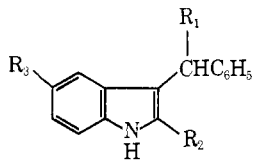
Table III *(Continued)*



Compd no.	R	R_1	R_2	R_3	Compd concn, μg/ml, followed by microorganism designation
36	5-CH_3	H	H	CHO	100, ck I
37	H	H	H	CHO	200, cm XII XX XXII XXV XXVI
38	H	H	CH_2OH	H	200, abd III IV IX X XII XXV
39	H	H	H	CH_2OH	200, ab IV IX X XII XVII

[a] F. Piozzi and C. Fuganti, *Ann. Chim. (Rome)*, **57** (5), 486 (1967) [*Chem. Abstr.*, **67**, 64295r (1967)]. [b] A. Kalir and Z. Pelah, *Isr. J. Chem.*, 4 (4), 155 (1966) [*Chem. Abstr.*, **66**, 55328f (1967)]. [c] K. Schofield and R. S. Theobald, *J. Chem. Soc.*, 796 (1949). [d] J. I. DeGraw, J. G. Kennedy, and W. A. Skinner, *J. Heterocycl. Chem.*, **3**, 67 (1966). [e]UpJohn Co., British Patent 781,390 (Aug 21, 1957) [*Chem. Abstr.*, **52**, 3866g (1958)]. [f]E. Shaw, *J. Amer. Chem. Soc.*, **77**, 4319 (1955). [g]Merrill E. Speeter (to UpJohn Co.), U. S. Patent 2,814,625 (Nov 26, 1957) [*Chem. Abstr.*, **52**, 11949e (1958)]. [h] L. B. Shagalov, N. P. Sorokina, and N. N. Suvorov, *Zh. Obshch. Khim.*, **34**, 1592 (1964) [*Chem. Abstr.*, **61**, 5596c (1964)]. [i]D. H. Lively, M. Gorman, M. E. Haney, and J. A. Mabe, *Antimicrob. Agents Chemother.*, 462 (1966). [j]J. R. Piper and F. J. Stevens, *J. Heterocycl. Chem.*, **3**, 95 (1966). [k]W. R. Boehme, *J. Amer. Chem. Soc.*, **75**, 2502 (1953). [l]A. S. Katner, *Org. Prep. Proced.*, **2** (4), 297 (1970). [m]J. V. Braun and O. Bayer, *Ber.*, **58**, 387 (1925). [n]R. Rothstein and B. N. Feitelson, *C. R. Acad. Sci.*, **243**, 1042 (1956). [o]B. Heath-Brown and P. G. Philpott, *J. Chem. Soc.*, 7165 (1965).

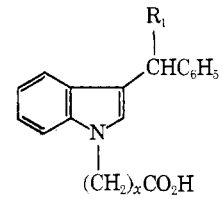
Table IV



Compd no.	R_1	R_2	R_3	Mp, °C	Yield, %[a]
40	C_6H_5	H	Br	147–148	**73**
41	C_6H_5	CO_2H	Cl	278 dec	62
42	C_6H_5	CO_2H	H	226 dec	68
43	C_6H_5	CO_2H	OH	240 dec	74
44	2-$CH_3OC_6H_4$	H	CO_2H	208	34
45	C_6H_5	CO_2H	$C_6H_5CH_2O$	182 dec	
53	2-$CH_3OC_6H_4$	H	H	160–161	62
54	4-$CH_3OC_6H_4$	H	H	115–117	38
70	4-$(CH_3)_2NC_6H_4$	H	H	158	**32**
75	C_6H_5	CH_3	Cl	181	12

[a] Yields are of analytically pure material.

Table V



Compd no.	R_1	x	Mp, °C	Yield, %
46	4-ClC_6H_4[a]	1	205	71
47	4-ClC_6H_4	1	185	66
48	4-FC_6H_4 .	1	185	60
49	C_6H_5	1	201	84
50	C_6H_5	2	161	69
55	3,4,5-$(CH_3OC_6H_2)_3$	1	100–102	24

[a] 4,4′-Dichlorodiphenylmethyl-1-indoleacetic acid.

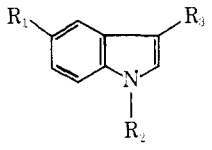
bles IV and VI) did not contribute to activity, but 2-(diphenylmethyl)-3-[2-[*N*-(diphenylmethyl)amino]ethyl]indole (**72**, Table VII) at 10 and 100 μg/ml was active against bacteria. Unlike **72**, the other derivatives that were substituted with two diarylmethyl groups (**73**, **74**, Table VI) were completely inactive at 200 μg/ml.

The slight activity found with 5-chloro-3-(diphenylmethyl)-2-methylindole (**75**, Table IV) was unexpected since the parent 5-chloro-2-methylindole (**25**, Table III) had a broad spectrum of activity. Compounds **76** and **77** (Table VII) were only slightly active and **78** had no activity at 100 μg/ml. These results were expected since the parent compounds were inactive and belonged to groups of indoles that showed a low incidence of activity.

Compounds **40**, **41**, **44**, **46–49**, **51**, **54**, **55**, **60**, **66**, **68**, and **77** (Tables IV–VIII) at 500–2000 μg/ml inhibited the growth of several protozoa, but their effectiveness was not of practical importance.

The effects of the (diarylmethyl)indoles on the behavior of mice were evaluated by Kattau, *et al.*, according to procedures previously described.[12] Suspensions of the compounds were administered intraperitoneally to male white mice (Cox) weighing 16–20 g. Three mice were given each standard dose and observed for changes in behavior, appearance, and response to certain stimuli. The standard doses used were 10, 25, 50, 100, 300, 400, 800, and 1600 mg/kg. The behavior and toxicity are given in Table IX. The mice were observed for 4 days. All deaths were considered to be drug related. The largest dose size that was not lethal is shown by the smallest number in the toxicity column (Table IX). When a range is given, the largest dose was lethal to one out of three mice. Only relative toxicities are derived from these numbers. Compounds **42**, **44–48**, **51**, and **55** were the most toxic of the series with a lethal dose range of 25–200 mg/kg. They are all acid derivatives, with carboxy groups in the 2 or 3 position or an acetic acid group in position 1. Compounds with a 2- or 5-carboxy group (**41**, **74**) or an acetic acid group in positions 1 or 3 (**51**, **65**) had a lethal range of 200–400 mg/kg. The least toxic (diarylmethyl)indoles with a lethal range of 400–1600 mg/kg include propionic and butyric acids (**58**, **69**, **50**) and neutral or basic derivatives (**40**, **53**, **54**, **57**, **60**, **62–64**, and **78**). The only recognizable behavioral effects, other than toxic reactions, were weak to very weak central nervous depression.

Table VI



Compd no.	R_1	R_2	R_3
51	H	CH_2CO_2H	$C_{13}H_9O$[a]
56	NO_2	H	$C_{14}H_{11}O_2$[b]
57	CH_3O	H	$C_{16}H_{17}O_3$[c]
59	H	H	$(C_6H_5)_2CH$
60	H	H	$C_{13}H_9O$[a]
61	H	CH_3	$(C_6H_5)_2CH$
62	H	H	$(C_6H_5)_3C$
63	H	H	$C_{20}H_{17}$[d]
64	H	H	$C_{21}H_{17}O$[e]
71	H	H	$C_{19}H_{16}NO$[f]
73	H	$C_{13}H_9O$[a]	$C_{13}H_9O$[a]
74	CO_2H	$(C_6H_5)_2CH$	$(C_6H_5)_2CH$

[a] Xanthen-9-yl. [b] α-(3,4-Methylenedioxyphenyl)benzyl. [c] α-(3,4,5-Trimethoxyphenyl)benzyl. [d] 1,1,2-Triphenylethyl. [e] β,β,β-Triphenylpropionyl. [f] α-Methyl-4-phenoxy-α-(1-pyridyl)benzyl.

The (diarylmethyl)indoles were screened for antiviral activity by D. C. Delong and associates of the Lilly Laboratories. This screening test is designed to detect compounds that interfere with any phase of the virus life cycle and the procedure is essentially the agar diffusion test described by Herrmann, *et al.*[13]

The size of the zone of inhibition, measured in millimeters, is only a reflection of the ability of a chemical to diffuse through agar. A grading system is used to distinguish between compounds that show incomplete protection and those that give complete protection. A grade of 4+ indicates complete cell protection within the measured zone of inhibition; a grade of 3+ indicates that there are isolated areas of virus cytopathology within the zone; a grade of 2+ indicates a general distribution of viral cytopathology; and a grade of 1+ indicates well-developed microplaques in the zone.

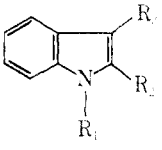
Compounds **42**, **44**, **47**, **48**, and **50** had antiviral activity (Table X);† however, with the exception of **50**, these compounds were the most toxic in the (diarylmethyl)indole series. The antiviral activity therefore may be a nonspecific toxic reaction.

Compounds were tested for antiinflammatory activity by N. B. Dininger and C. E. Brown and the procedure was essentially the same as that reported by Winter.[14] The results in Table IX are expressed as per cent inhibition, using butazolidine as the standard. The most active compounds, **46**, **50**, **55**, **56**, **60**, **75**, **76**, **63**, **64**, **74**, and **72**, inhibited the inflammation in the rat's paw by 20–43%, when administered (20–100 mg/kg) either subcutaneously or orally on the day prior and the morning of the test day.

In vitro antispasmotic activity testing conducted by J. W. Aiken and J. E. Waddell was that commonly used in pharmacological screening methods. The appropriate muscle strip was suspended in oxygenated Tyrode's solution and muscle responses to agonist and antagonist were recorded electronically through an isotonic myograph. Contractions of rat stomach fundus strips were induced by serotonin and contractions of guinea pig ileum strips were induced by histamine or methacholine. Following contact of the compound with the muscle for a period of 5 or 10 min, the agonist was readministered and the muscle contraction was measured. The antispasmotic activity is reported as the concentration of compound in μg/ml of solution required to inhibit muscle contraction by 50%. The antispasmotic activities listed in Table XI† are neither outstanding nor interesting.

† See paragraph at end of paper regarding supplementary material.

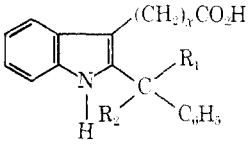
Table VII



Compd no.	R_1	R_2	R_3
52	CH_2CO_2H	$C_{13}H_9O$[a]	$C_{13}H_9O$[a]
65	H	$(C_6H_5)_2CH$	$(CH_2)_2CO_2H$
66	H	$(C_6H_5)_2CH$	$(CH_2)_3CO_2H$
72	H	$(C_6H_5)_2CH$	$C_{15}H_{16}N$[b]
76	H	$C_{13}H_9O$[a]	CH_3CO
77	CH_3	CO_2H	$C_{14}H_{11}O_2$[c]
78	H	$(C_6H_5)_2CH$	$C_5H_7NO_3$[d]

[a] Xanthen-9-yl. [b] Diphenylmethylaminoethyl. [c] 4-Carboxyphenylbenzyl. [d] (2-Acetamido-2-carboxy)ethyl.

Table VIII



Compd no.	x	R_1	R_2	Mp, °C	Yield, %
58	3	$3,4,5\text{-}(CH_3OC_6H_2)_3$	H	219	73
67	1	C_6H_5	H	233–235	75
68	1	$2\text{-}CH_3OC_6H_4$	H	192–194	19
69	3	C_6H_5	C_6H_5	220	3

Chemistry. (Diarylmethyl)indoles, Tables IV–VIII, were obtained by the carbonium ion alkylations of selected indole derivatives. Alkylations occurred preferentially at the 3 position but when that position was occupied, substitutions took place at the 2 position. Diphenylmethanol gave better yields than the substituted diphenylmethanols. For example, a good yield of 5-bromo-3-(diphenylmethyl)indole was obtained with 5-bromoindole and diphenylmethanol, but 5-bromoindole gave intractable tars with both 4-chloro- and 2,4-dichlorodiphenylmethanol. Alkylations of 1-indoleacetic acid occurred readily with 4-chloro-, 4-fluoro-, and 4,4′-dichlorodiphen-

Table IX. Biological Activities of Diarylmethylindoles

Compd no.	Mouse toxicity, mg/kg	Antiinflam act., % (mg/kg)	Antimicrobial act., MIC organism
40	>400	27.9 (20)	0.78, ae; 10, bcfg; 100, VI XVII
41	200–400	13.1 (100)	3.12, a; 6.25, c; 10, bcfg; 10, IV VI XXII XXIV; 100, hi IX XXV XXVII
42	25–50	29.3 (100)	100, a–c ef
43	300–1000	6.5	100, a–c ef
44	100–200	15.9 (100)	<10, a–g; 100, VI
45	100–300	4.8 (50)	<10 a–g I VI
46	100–400	13.9 (20), 35.2 (100)	12.5, a–g VI; 100, IV IX XXII XXVIII
47	50–100	30.4 (100)	12.5, a–g; 100, IV VI VII IX XVII XXVIII
48	25–50	14.9 (100)	10, b; 25, a–g; 100, I VI XXIV
49	400	17.7 (100)	<10, VI
50	800–1600	11.5 (20), 39.7 (100)	<10, b; 100, acg VI
51	<200	0	<10, c; 100, abg IV VI XXIV
52	300–1000	10.0 (50)	<10, a–g I IX XXIV XXV; 100, VI
53	>1600	20.5 (50)	None
54	>400	24.7 (100)	None
55	50–100	29.5 (100)	None
56	>400	39.9 (100)	None
57	>1000	17.1 (50)	None
58	>400	39.8 (100)	None
59	>400	45.6 (20)	<10, c
60	>1000	26.5 (50)	<10, bc XXVII; 100, VI VII
61		25.6 (50)	
62	>400	0 (100)	<10, c
63	>400	43.5 (100)	100, VI
64	>400	18.6 (20)	None
65	200–400	0	
66	>400	0	100, ab VI
67	>400		
68	200–400		100, a–c hik
69	>400	24.2 (100)	100, a
70	>1600	5.6 (100)	100, VI XXII
71	>1000	10.9 (50)	100, VI
72	>400	22.1	<10, cg; 100, abef
73	>1000	0	<10, a–g I IX XXIV XXV; 100, VI
74	>400	29.9 (100)	None
75		33.9 (20)	100, c
76	>1000	23.8 (50)	100, VI
77	400–800	0	100, a–c
78	400–1600	0	None

ylmethanol to give the corresponding 3-diarylmethyl-1-indoleacetic acids (46–48, Table V). This indicated that failures with 5-bromoindole were due to decomposition reactions of the indole rather than failure of the diarylmethanols to form stable carbonium ions. Also, the addition of a catalytic amount of boron trifluoride etherate to 5-bromoindole and the diarylmethanol in hot acetic acid only speeded decomposition. The reaction was slow between 1-indolepropionic acid and diphenylmethanol, and extending the reaction time from 8 to 24 hr increased the yield of 3-(diphenylmethyl)-1-indolepropionic acid from 6 to 69%. Purification difficulties were responsible for the low yield of 3-[4-(dimethylamino)diphenylmethyl]indole. Alkylations of 3-indoleacetic acid and 3-indolebutyric acid gave good yields of 2-(diphenylmethyl)-3-indoleacetic acid and 2-(3,4,5-trimethoxydiphenyl)-3-indolebutyric acid. Tritylation of 3-indolebutyric acid with triphenylmethanol in hot acetic acid gave a very small yield of 2-(triphenylmethyl)-3-indolebutyric acid. Most of the indolebutyric acid was recovered unchanged along with triphenylmethane.

Because of our interest in the (diarylmethyl)indoles studied here, we continued investigating the carbonium ion reactions with indoles and other heterocyclic compounds. The compounds listed in Tables VI and VII were borrowed from this separate study and their chemistry is reported in another communication.[15]

Experimental Section

Diarylmethylation of Indole Derivatives, Compounds 40–50, 53, 55, 58, 67–70, 75 (Tables IV, V, VIII). The indole (0.1 mol) and an equal molar amount of benzhydrol or the substituted benz-

hydrol were dissolved in 100–200 ml of glacial acetic acid. The solution was heated at refluxing temperature for 8 hr. A sample of the solution was added to cold water. The unreacted diphenylmethyl acetate present in the precipitate from incomplete reactions gave oils that would not solidify. Completed reactions yielded solid products or oils that eventually crystallized. These products also gave mainly one spot when developed on thin-layer plates. The cooled reaction mixture was added to cold water and the resulting solid was collected and washed with cold water. The products were dissolved in hot ethyl acetate and *n*-hexane was added until incipient turbidity. The compounds usually crystallized but a few intensely colored compounds did not. The latter required clarification with carbon, time, and concentration changes to initiate crystallization. All yields are reported for analytically pure compounds.

Compound **50** [Table V, 3-(diphenylmethyl)-1-indolepropionic acid] failed to crystallize and was chromatographed on a silica column with 1% acetic acid in ethyl acetate.

Structures of the alkylation products were established by elemental analysis and pmr spectra. The purity of the compounds was checked with thin-layer chromatography but the melting points were not calibrated.

5-Hydroxy-3-(diphenylmethyl)-2-indolecarboxylic Acid (43, Table IV). 5-Benzyloxy-3-(diphenylmethyl)-2-indolecarboxylic acid (8.7 g or 0.023 mol) in 500 ml of alcohol was heated at 40–50° with hydrogen at 50 psi over 5% Pd/C catalyst in a Parr high-pressure autoclave until there was no further drop in hydrogen pressure (3 hr). The solution was filtered and evaporated; the resulting product was crystallized from a mixture of ethyl acetate and *n*-hexane.

Supplementary Material Available. Elemental analysis for compounds **40–75** and Tables II, X, and XI will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JMED-74-1298.

References

(1) A. Lewis and R. G. Shepherd, "Medicinal Chemistry," 3rd ed, part 1, A. Burger, Ed., Wiley-Interscience, New York, N. Y., 1970, p 448; T. S. Osdene, p 662.
(2) A. M. Akkerman and H. Veldstra, *Recl. Trav. Chim. Pays-Bas*, **73**, 629 (1954).
(3) A. M. Akkerman, D. K. deJongh, and H. Veldstra, *Recl. Trav. Chim. Pays-Bas*, **70**, 899 (1951).
(4) E. Shaw and D. W. Woolley, *J. Pharmacol. Exp. Ther.*, 111, 43 (1954).
(5) W. T. Comer and A. W. Gomoll, "Medicinal Chemistry," 3rd ed, part 2, A. Burger, Ed., Wiley-Interscience, New York, N. Y., 1970, p 1038.
(6) V. Erspamer, *Science*, **121**, 369 (1955).
(7) D. W. Wylie and S. Archer, *J. Med. Pharm. Chem.*, **5**, 932 (1962).
(8) C. L. Zirkle and C. Kaiser, ref 5, p 1459.
(9) J. Offermeier and E. J. Ariens, *Arch. Int. Pharmacodyn.*, **164**, 192, 216 (1966).
(10) L. S. Goodman and A. Gilman, "The Pharmacological Basis of Therapeutics," 4th ed, Macmillan, New York, N. Y., 1970, p 337.
(11) I. S. Johnson, P. J. Simpson, and J. C. Cline, *Cancer Res.*, **22**, 617 (1962).
(12) (a) R. C. Rathbun, J. K. Henderson, R. W. Kattau, and C. E. Keller, *J. Pharmacol. Exp. Ther.*, **122**, 64A (1958); (b) R. C. Rathbun and I. H. Slater, *Psychopharmacologia*, **4**, 114 (1963).
(13) E. C. Herrmann, Jr., J. Gabliks, C. Engle, and P. L. Perlman, *Proc. Soc. Exp. Biol. Med.*, **103**, 625 (1960).
(14) C. A. Winter, *et al.*, *Proc. Soc. Exp. Biol. Med.*, 111, 544 (1962).
(15) C. W. Whitehead and C. A. Whitesitt, *J. Org. Chem.*, submitted for publication.

Selective Binding of Metal Ions to Macromolecules Using Bifunctional Analogs of EDTA

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The synthesis of 1-(*p*-benzenediazonium)ethylenediaminetetraacetic acid, the coupling of this compound to proteins, and the binding of radioactive metal ions to the protein-bound chelating groups are described. This procedure provides a novel approach to the preparation of radiopharmaceuticals, permitting the separation of synthetic organic chemistry from radiochemistry. Azoproteins labeled with indium-111 are relatively stable *in vivo* and potentially useful for the detection and localization of tumors. Other chelating agents derived from 1-(*p*-aminophenyl)ethylenediaminetetraacetic acid may permit new applications of a variety of metal ions with useful physical properties to studies of biological systems.

Polyaminocarboxylate chelating agents such as ethylenediaminetetraacetic acid form stable complexes with the ions of many heavy metals. Since these metal ions possess a variety of useful spectroscopic and radioactive properties, the preparation of chelating agents whose complexes interact, in some selected manner, with biological macromolecules may lead to new applications of metal ions as probes of biological systems.

As an intermediate in the preparation of molecules with the dual properties mentioned above, 1-(*p*-aminophenyl)ethylenediaminetetraacetic acid (**6**, Scheme I) has several advantages. In principle, the aromatic amino group of this compound can be acylated, alkylated, or otherwise modified to form either biologically active derivatives or covalent labeling reagents. The specific interaction between 1-(*p*-nitrophenyl)ethylenediaminetetraacetic acid