stability of the aziridine ring is well documented,[15] there was some doubt as to whether the relatively high activity observed with compounds IIc and IId was due to the intact molecule or to one of the hydrolysis products. A study of the stability of these two compounds under *in vitro* testing conditions in phosphate buffer (pH 7.4), containing not more than 4% *p*-dioxane, indicated that the isopropyl derivative was stable whereas the phenethyl derivative underwent slight hydrolysis as detected by thin layer chromatography using ethyl acetate–benzene (1:3) as solvent.

Experiments were designed to determine whether the MAO-inhibitory action of 1-(2-phenethyl)-2-phenylaziridine was due to the intact molecule or a hydrolysis product. Both 2-(2-phenethyl)amino-1-phenylethanol and 2-(phenethyl)amino-2-phenylethanol were tested along with 1-(2-phenethyl)-2-phenylaziridine for *in vitro* MAO inhibition. Although the results indicated that 2-(2-phenethyl)amino-1-phenylethanol has considerable activity at 5×10^{-4} *M* concentration, its ID_{50} was found to be 9.25×10^{-5} *M*. This indicated that the above amino alcohol, in the amounts formed during *in vitro* testing could not be solely responsible for the MAO inhibition observed with compound IId. Thin layer chromatograms with a solution of Id (9.25×10^{-5} *M*) and of IId which gave approximately corresponding *in vitro* activity showed that, whereas the amino alcohol was easily detectable on the plates, the parent aziridine showed only a very faint spot of hydrolysis product after subjecting the two to the *in vitro* testing conditions.

It is possible that the inductive effect of an alkyl substituent would serve to increase the availability of the unshared pair of electrons on the aziridine nitrogen, making it favored as a center of high nucleophilic reactivity.[16] This does not, however, explain why compound IId is considerably more active than compound IIb. The inductive effect should be about the same from both substituents, which leaves a bulk effect as a possible explanation. The compounds which are most active (IIc and IId) have in common at the 1-position, nonpolar, hydrocarbon moieties which have considerable bulk.

Compound III which contains a polar group also shows MAO-inhibition activity. However, the observation that IV and V showed very little activity as compared to III leads to the conclusion that even in the series of polar substituents in the 1-position, greatest activity is shown by the compounds that have a bulky substituent.

Although 2-amino-1-phenylethanol is a good substrate of MAO,[17] no phenethylamine derivatives containing groups larger than methyl or dimethyl on the nitrogen have been found to be good substrates of MAO. One may speculate that due to the presence of the phenethyl unit, compounds Ic and Id are able to establish their presence near the active site of MAO and thus may partially prevent the enzymatic oxidation of the substrate. Here again it would seem that the large hydrocarbon substituent of the 1-position is responsible for inhibitory activity.

(15) J. E. Early, C. E. O'Rourke, L. B. Clapp, J. O. Edwards, and B. C. Lawes, *J. Am. Chem. Soc.*, **80**, 3458 (1958).

(16) B. Belleau and J. Moran, *J. Med. Pharm. Chem.*, **5**, 215 (1962).

(17) N. Weiner, *Arch. Biochem.*, **91**, 182 (1960).

Synthesis of Chelating Compounds to Be Used as Potential Bone Seekers[1,2]

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A number of new derivatives of iminodiacetic acid, of the general formula $RN^{+}H(CH_2COOH)CH_2COO^{-}$ have been prepared for evaluation as potential bone seekers. Radical R stands for a chain containing, *inter alia*, an oxygen atom belonging to a hydroxy, carbonyl, amide, carboxyl, or ether function. In this way, tridentate or quadridentate chelating agents are formed. These compounds were prepared mainly by introducing the iminodiacetic acid group *in toto* into different molecules by the action of various alkyl halides on iminodiacetic acid dimethyl ester in nonpolar solvents, followed by saponification and precipitation of the imino acids at their isoelectric points. In order to study their biological behavior, the imino acids were labeled with one of the radiohalogens F^{18}, Br^{82}, or I^{131}. The labeling methods were based on exchange reactions, direct halogenation, or recoil labeling. Acid dissociation constants of the imino acids and the chelate stability constants of the corresponding anions with divalent metal ions were determined potentiometrically. With calcium ions, the major metallic constituent of bone mineral, each of the amino acids forms a single stable 1:1 metal chelate, its stability ranging from $\log K_{S_1} = 3$ to $\log K_{S_1} = 5$ ($t = 30°$, $\mu = 0.100$). Biological studies have proved that the affinity of the different synthetic imino acids for bone can be correlated with their chelating ability with calcium ions.

Bone seekers as a group comprise a heterogeneous list which by broad definition includes any substance localizing in the skeleton.[4] Most of the research work on bone seekers has been concerned with radioactive elements, because of the radiation hazard arising from the deposition of such elements in the skeleton, and because they can be used to study the physiological activities of bone, qualitatively and quantitatively. Bone-seeking radioelements have also found extensive use in the study of both localized lesions in bone (*e.g.*, fractures and primary or metastatic neoplasms) and metabolic disorders of the skeleton (*e.g.*, Paget's

(1) This investigation was supported by the Israel Atomic Energy Commission, Contract 49-04.

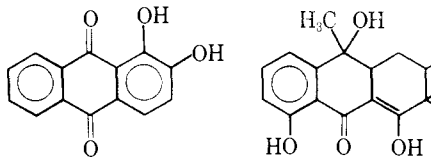
(2) Part of a thesis submitted by G. Shtacher to the Senate of the Hebrew University, Jerusalem, 1965, in partial fulfillment of the requirements for the Ph.D. degree.

(3) The Hospital for Special Surgery, New York, N. Y.

(4) P. S. Chen, Jr., A. R. Terepka, and H. C. Hodge, *Ann. Rev. Pharmacol.*, **1**, 369 (1961).

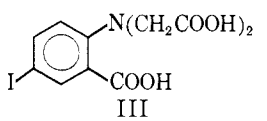
disease).[5] In contrast to mineral bone seekers, relatively little is known about organic bone seekers.

Organic compounds which have, so far, been shown to deposit in bone mineral are (a) alizarin (I) and related anthraquinone dyes which stain new bones by forming calcium lakes (the early studies on bone growth using alizarin were based on the red color of the skeleton laid down during feeding of the dye[6]), (b) tetracyclines (II) which complex calcium in solution and have been shown to localize in bone mineral by ultraviolet fluorescence techniques,[7] and (c) N-(2-carboxy-4-iodophenyl)iminodiacetic acid (III) which accumulates in growth areas of bone and was assumed to act as a tetradentate chelating agent.[8]



I

II



III

The exact mechanism of localization of compounds I–III has not been elucidated completely, but since the feature common to the three organic bone seekers mentioned above is their chelating property, it has been assumed that their deposition in bone is the result of chelate formation with calcium ions at the surface of bone crystals. Stability constants for these chelates with calcium and magnesium ions were recorded as shown below in Table I.

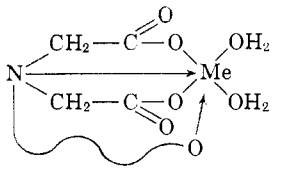
TABLE I

Ligand	Metal ion	Stability constant (log value)	
N-(2-Carboxyphenyl)-iminodiacetic acid	Ca^{2+}	5.06	(μ = 0.10, t = 20°)[a]
	Mg^{2+}	3.91	
Tetracycline (III)	Ca^{2+}	5.52	(μ = 0.11, t = 30°)[b]
	Mg^{2+}	4.85	

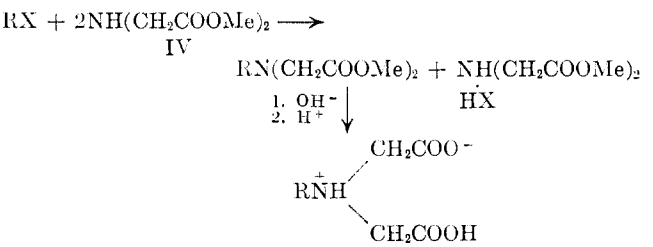
[a] G. Schwarzenbach, A. Willi, and R. O. Bach, *Helv. Chim. Acta*, **30**, 1303 (1947). [b] D. C. Maxwell, P. J. A. Smith, and S. P. Wilford, *Nature*, **198**, 577 (1963).

In order to study the relationship between chelating property and affinity for bone mineral, it was of interest to synthesize a number of new chelating agents, forming calcium chelates with stability constants of the same order of magnitude as compounds II and III, and to study their affinity for bone.

Chemistry.—For biological research purposes, interest was focused on calcium chelates with a stability constant of approximately log 5. A survey of the literature[9] shows that only ligands of the aminopolycarboxy type satisfy this requirement. These ligands include in their structure the iminodiacetic acid group, in combination with carboxy, hydroxy, or methoxy groups. Phenyl-substituted iminodiacetic acids were therefore synthesized, carrying either a carbonyl or ether oxygen in the side chain on the nitrogen, thus forming potential quadridentate complexing agents.



The phenyl ring was used as a carrier for the radioactive halogens F^{18}, Br^{82}, or I^{131} which served as tracers. The aromatic carbon–halogen bond, due to its relative stability, is not readily cleaved *in vivo*.[10] Of the various methods for preparing iminodiacetic acid derivatives,[11] the most frequently used was the alkylation of iminodiacetic acid dimethyl ester (IV)[12] with different alkyl halides (mostly bromides), followed by saponification of the resulting tertiary imino esters and isolation of the free imino acids. This method was found most convenient for the following reasons.



(a) The starting materials, both the various alkyl halides and the iminodiacetic acid dimethyl ester, are easily obtainable in any amount and in good yields. (b) The alkylation of iminodiacetic acid dimethyl ester proceeds smoothly in yields of 80–100% according to the reactivity of the halogen. (c) The progress of the alkylation reaction can be followed easily by weighing the hydrohalide salt of the iminodiacetic acid dimethyl ester, $HX \cdot NH(CH_2COOMe)_2$, which separates from the reaction mixture (nonpolar solvent). The free imino ester is recovered in almost quantitative yield from its salt. (d) The imino methyl esters are easily saponified under mild conditions to the corresponding imino acids in quantitative yield.

Stability Constants of Metal Complexes with Certain Amino Dicarboxylic Acids.—The acid dissociation constants of N-benzyl-, N-phenacyl-, N-(β-phenoxyethyl)-, and N-(*p*-bromoacetanilido)iminodiacetic acids, and of N-benzylaspartic acid (*dl*), as well as the chelate-stability constants of the corresponding anions with divalent Mg, Ca, and Ni at 30° and ionic strength 0.1 *M* (KCl) were determined potentiometrically. Experimental data to be published elsewhere[13] indicate that for the four imino acids, the relative stability of the metal chelates is Ni > Ca > Mg, as could be expected. The contribution of the oxygen-donor atom belonging to the keto group of N-phenacyl-, or to the amido group of N-(*p*-bromoacetanilido)iminodiacetic

(5) F. C. Mclean and A. M. Bloly, "Radiation, Isotopes and Bone," Academic Press Inc., New York, N. Y., 1964, pp 187–198.

(6) J. Belehier, *Phil. Trans. Roy. Soc. London*, **39**, 287, 299 (1736).

(7) R. A. Milch, D. P. Rall, and J. E. Tobie, *J. Bone Joint Surg.*, **40A**, 897 (1958).

(8) M. Anbar, J. Aviad, R. Rein, and S. Schorr, *Experientia*, **16**, 443 (1960).

(9) S. Chaberek and A. E. Martell, "Organic Sequestering Agents," John Wiley and Sons, Inc., New York, N. Y., 1959.

(10) B. Chenoweth and L. P. McCarthy, *Pharmacol. Rev.*, **15**, 673 (1963).

(11) T. Weiland and H. Soll in "Methoden Der Organischen Chemie" (Houben-Weyl), Vol 11/2, Georg Thieme Verlag, Stuttgart, 1958, pp 405–416.

(12) J. V. Dubsky and Ch. Graenacher, *Ber.*, **50**, 1692 (1917).

(13) G. Shtacher, *J. Inorg. Nucl. Chem.*, in press.

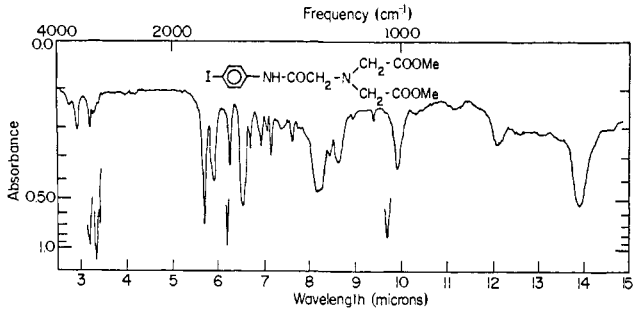


Figure 1.—Infrared spectrum of N-(*p*-iodoacetanilido)iminodiacetic acid dimethyl ester (XIX) in $CHCl_3$ (10%).

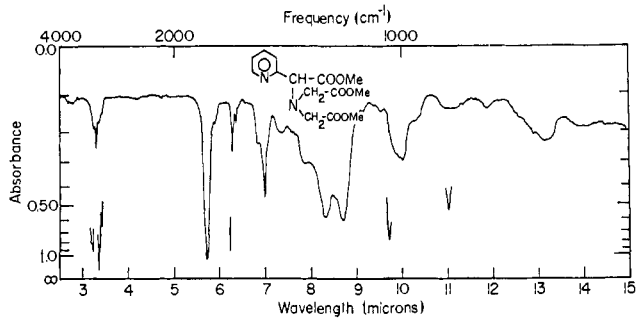


Figure 2.—Infrared spectrum of C-(2-pyridyl)nitrilotriacetic acid trimethyl ester (XXI) (neat).

acids, is appreciable in coordinating calcium ions. Both ligands behave as tetradentate chelating agents in their 1:1 complexes with calcium. Each of the imino acids forms a single stable 1:1 metal chelate with calcium ions, the stability ranging from log $K_{S_1} \sim 3$ to log $K_{S_1} \sim 5$. N-Benzylaspartic acid, on the other hand, does not complex calcium ions to any measurable extent.

Biological Evaluation.—Preliminary *in vivo* studies[14] on the affinity of the different chelating agents for bone have shown that only those acids which are able to complex calcium are selectively localized in bone tissue. Various bone tissues showed different affinities for the chelating agents, the highest accumulation being in the region of the epiphysial plates, which are sites of very active bone formation. The pattern of uptake of the organic bone seekers and of fluoride ion (a well-known mineral bone seeker) was similar under physiological and nonphysiological conditions. All compounds were cleared from the body very rapidly, mainly through the kidneys; their concentration in bone dropped at a much slower rate. The compounds are metabolically inert, and no toxic effects were observed on young rats even at massive doses of 500 mg/kg of body weight.

Experimental Section[15]

Titrimetric analysis and molecular weight determination of the imino esters and the imino acids were carried out as shown in Table II.

TABLE II

Compd	Solvent	Titrant	Indicator
Imino acids	Water	NaOH	Methyl red
	Absolute ethanol	$NaOCH_3$	Thymol blue
	Glacial acetic acid	$HClO_4$	Methyl violet
Imino esters	Glacial acetic acid, or absolute dioxane	$HClO_4$	Methyl violet
Hydrochloride or hydrobromide salts of imino esters	Absolute methanol or ethanol	$NaOCH_3$	Thymol blue

In the general method used for preparing iminodiacetic acid derivatives, aralkyl bromide (1 mole) and iminodiacetic acid dimethyl ester (2 moles) were dissolved in dry benzene or dioxane. The reaction mixture was protected from moisture and CO_2 (soda lime tube). In the case of active bromides (*e.g.*, benzyl and phenacyl), the hydrobromide salt of iminodiacetic acid dimethyl ester (IV·HBr) began to separate as soon as the two reactants were brought together at room temperature. The reaction mixture was refluxed gently until there was no more precipitation of the hydrobromide salt (usually about 1 hr) after which time the mixture was cooled and filtered, and the hydrobromide salt was washed with dry ether. The filtrate was concentrated *in vacuo* and the residue, the product, was distilled under reduced pressure or crystallized. The free iminodiacetic acid dimethyl ester was recovered from its hydrobromide salt by treating a suspension of the salt in $CHCl_3$ with a slight excess of triethylamine or by treating a suspension of the salt in ether with a saturated solution of K_2CO_3 in the cold.

The pure *t*-imino esters (1 mole) were usually saponified with NaOH (~2 *N*, 2.4 moles) in methanol-water which gave a homogeneous phase. The saponification was complete in 0.5 hr at room temperature, after which time the reaction mixture was extracted with ether or ethyl acetate and acidified carefully in the cold (HCl, ~3 *N*) to the isoelectric point of the imino acid. The precipitated imino acid was filtered, washed with water, and crystallized from a suitable solvent. Imino acids which were highly soluble in water were obtained from their methyl esters by saponification with LiOH (~2 *N*), followed by acidification to the isoelectric point with HCl (~3 *N*) and addition of tetrahydrofuran to the aqueous solution. The imino acids were thus precipitated while the LiCl salt remained in solution.

The parent compound, **iminodiacetic acid dimethyl ester (IV)**, was prepared according to Dubsky and Graenacher;[12] yield 35%, bp 62° (0.10 mm), 95.5° (0.80 mm), 134.5° (20.0 mm), $n^{26}D$ 1.4390, lit.[12] bp 126° (33 mm). It was further characterized as the hydrobromide salt, mp 191–192° dec (dry methanol).

Anal. Calcd for $C_6H_{12}BrNO_4$: C, 29.76; H, 5.00; Br, 33.01; N, 5.79; mol wt, 242.08. Found: C, 29.76; H, 4.96; Br, 33.10; N, 5.78; mol wt, 241.00.

The analyses and physical constants of the N-substituted iminodiacetic acids and esters prepared are given in Tables III and IV. The infrared spectra of two compounds (XIX and XXI) are shown in Figures 1 and 2.

***m*-Bromo-*p*-methoxyphenacyl bromide**, required for the synthesis of XIV, was prepared by bromination of *m*-bromo-*p*-methoxyacetophenone[16] in $CHCl_3$ with bromine; yield 92%, mp 114–115° (MeOH).

Anal. Calcd for $C_9H_8Br_2O_2$: C, 35.10; H, 2.61; Br, 51.90. Found: C, 35.12; H, 2.70; Br, 52.28.

The above compound has been prepared by the Friedel–Crafts synthesis (bromoacetyl chloride and *o*-bromoanisole);[17] mp 105° The yield and analysis were not given.

β-(*p*-Fluorophenoxy)ethyl bromide, for the synthesis of XVI, was prepared in 50% yield from *p*-fluorophenol and ethylene bromide in NaOH solution. The progress of the reaction was

(14) G. Shtacher and M. Anbar, *J. Pharmacol. Exptl. Therap.*, in press.

(15) All melting points and boiling points are uncorrected. Melting points were determined on a Fisher-Johns melting point apparatus. Infrared spectra were recorded on a Perkin-Elmer Infracord spectrophotometer Model 137, employing CCl_4 or $CHCl_3$ solutions, with NaCl optics, calibrated by polystyrene film. Paper chromatography of the imino acids was done by the ascending technique on Whatman No. 1 paper. The solvent system was 1-butanol-acetic acid-water (780:100, saturation). The chromatograms were developed for about 22 hr at room temperature (*ca.* 20°), and by that time the solvent front had moved about 31 cm. The chromatograms were dried (70°) and the spots were detected by spraying with bromocresol green indicator dissolved in 1-butanol. The imino acids appeared as bright yellow spots on gray-blue background. Thin layer chromatography was carried out on silica gel G (E. Merck, Germany). The plates were prepared using a Desaga applicator set for a thickness of 0.25 mm. The compounds were detected by iodine vapor. Microanalyses were performed by the microanalytical laboratory of the Weizmann Institute of Science under the direction of Mr. R. Heller.

(16) F. Kroehnke and E. Ellegast, *Ber.*, **86**, 1556 (1953).

(17) Ng. Hoan and Ng. Ph. Buu Hoi, *Compt. Rend.*, **224**, 1363 (1947).

TABLE III

N-SUBSTITUTED IMINODIACETIC ACID DIMETHYL ESTER DERIVATIVES

$RN(CH_2COOMe)_2$

No.	R	Form	Bp (mm) or mp, °C	n_D (°C)	Formula	Calcd, % C	H	Hal	N	Mol wt	Found, % C	H	Hal	N	Mol wt
V	Benzyl	Colorless oil	137 (0.45)	1.5009 (26)	$C_{13}H_{17}NO_4$	62.14	6.82		5.57	251.27	62.08	6.86		5.58	249.50
VI[a]	*p*-Fluorobenzyl	Colorless oil	134 (0.30)	1.4884 (28)	$C_{13}H_{16}FNO_4$	57.98	5.99		5.20	269.27	58.00	5.89		5.20	270.30
VII	*p*-Iodobenzyl	Yellowish oil	151–152 (0.15)	1.5530 (23)	$C_{13}H_{16}INO_4$	41.40	4.28	33.65	3.71	377.19	41.53	4.20	33.50	3.85	376.00
VIII	2-Hydroxy-5-bromobenzyl	Colorless needles[b]	49–50		$C_{13}H_{16}BrNO_5$	45.10	4.66	23.09	4.05	346.18	44.92	4.70	23.21	4.20	347.50
IX	*p*-Xylene-α,α'-di	Colorless needles[b]	90–91		$C_{20}H_{28}N_2O_8$	56.59	6.65		6.60	424.44	56.75	6.75		6.77	422.50
X	Phenacyl	Yellow viscous oil, gradually decomposes													
	Chloroplatinic acid salt	Orange rhombic crystals[c]	116–118		$C_{28}H_{38}Cl_6PtN_2O_{11}$	34.09	3.88	21.57 P_t:19.77	2.84		33.86	4.00	21.65 P_t:19.47	3.00	
XI[a]	*p*-Fluorophenacyl	Colorless needles,[b] gradually decomposes	65–66		$C_{14}H_{16}FNO_5$	56.56	5.42		4.71	297.28	56.64	5.44		4.62	298.50
XII	*p*-Bromophenacyl	Yellow, viscous oil, gradually decomposes	183–185 (0.30)		$C_{14}H_{16}BrNO_5$	46.94	4.50	22.31	3.91	358.19	46.65	4.43	22.27	3.98	360.00
XIII	*m*-Bromophenacyl	Yellow, viscous oil, gradually decomposes	175 (0.15)		$C_{14}H_{16}BrNO_5$	46.94	4.50	22.31	3.91	358.19	46.73	4.41	22.48	4.05	355.50
XIV[d]	*m*-Bromo-*p*-methoxyphenacyl	Colorless needles,[b] gradually decomposes	71–72		$C_{15}H_{18}BrNO_6$	46.40	4.67	20.58	3.61	388.24	46.54	4.60	20.75	3.50	390.50
XV	β-Phenoxyethyl	Yellowish oil	147 (0.03)	1.5140 (19)	$C_{14}H_{19}NO_5$	59.77	6.81		4.98	281.30	59.66	6.86		4.96	279.50
XVI[a]	β-(*p*-Fluorophenoxy)-ethyl	Colorless oil	164–166 (0.35)	1.4917 (29)	$C_{14}H_{18}FNO_5$	56.40	6.07		4.69	299.29	56.45	5.91		4.67	297.80
XVII	β-(*p*-Bromophenoxy)-ethyl	Colorless oil	157.5–160.5 (0.20)	1.5253 (28)	$C_{14}H_{18}BrNO_5$	46.68	5.04	22.19	3.89	360.21	46.87	5.17	22.35	4.01	362.00
XVIII	*p*-Bromoacetanilido	Colorless needles[b]	91–92		$C_{14}H_{17}BrN_2O_5$	45.01	4.59	21.41	7.51	373.21	45.20	4.45	21.43	7.63	370.50
XIX[e]	*p*-Iodoacetanilido	Colorless needles[b]	90.5–91.0		$C_{14}H_{17}IN_2O_5$	40.01	4.08	30.20	6.66	420.20	40.23	4.08	30.16	6.73	423.00
XXI[f]	2-Pyridylacetic acid, methyl ester, 2′-	Red viscous oil	149–151 (0.10)		$C_{14}H_{18}N_2O_6$	54.19	5.85		9.03	310.30	54.22	5.95		8.85	312.50

[a] No infrared absorption of phenolic OH in the region 2.70–3.00 μ. [b] Recrystallized from 2-propanol. [c] Recrystallized from methanol. [d] $\lambda_{max}^{ClO_3}$ (μ), 3.32 (aromatic CH), 5.74 (CO ester), 5.92 (CO ketone), 6.26, 6.68 (aromatic C=C). [e] $\lambda_{max}^{CHCl_3}$ (μ), 2.92 (NH amide stretching), 5.74 (CO ester), 5.95 (CO amide). [f] Thin layer chromatography indicated the presence of traces of colored impurities; λ_{max}^{neat} (μ), 3.31 (aromatic CH), 5.74 (CO), 6.32 (aromatic C=C), 8.31 (COC stretching of acetoxyl group).

TABLE IV: N-SUBSTITUTED IMINODIACETIC ACID DERIVATIVES $RN^{+}H(CH_2COO^-)(CH_2COOH)$

No.	R	Mp, °C[a]	Solvent	Formula	Calcd, % C	Calcd, % H	Calcd, % Hal	Calcd, % N	Mol wt	Found, % C	Found, % H	Found, % Hal	Found, % N	Mol wt
XXIII	*p*-Fluorobenzyl	191–193 dec	H_2O	$C_{11}H_{12}FNO_4$	54.77	5.01	7.87	5.80	241.21	54.82	5.14	8.13	5.65	242.00
XXIV	*p*-Iodobenzyl	215–216 dec	AcOH–H_2O	$C_{11}H_{12}INO_4$	37.84	3.46	36.35	4.01	349.13	37.72	3.50	36.40	4.01	349.00
XXV	2-Hydroxy-5-bromobenzyl	231.5–232.0 dec	DMF[b]–H_2O	$C_{11}H_{12}BrNO_5$	41.53	3.80	25.12	4.40	318.13	41.52	3.81	25.10	4.60	319.50
XXVI	*p*-Xylene-α,α'-di	248–250 dec	DMF–H_2O	$C_{16}H_{20}N_2O_8 \cdot 1H_2O$	49.74	5.74		7.25	386.37	50.03	5.72		7.31	384.00
XXVIII	Phenacyl	167–169 dec	H_2O	$C_{12}H_{13}NO_5$	57.37	5.22		5.58	251.23	57.50	5.19		5.47	252.00
XXIX	*p*-Fluorophenacyl	199–200 dec	DMF–H_2O	$C_{12}H_{12}FNO_5$	53.52	4.49	7.06	5.20	269.22	53.65	4.57	7.25	5.23	270.50
XXX	*p*-Bromophenacyl	185–186 dec	AcOH–H_2O	$C_{12}H_{12}BrNO_5$	43.65	3.66	24.21	4.24	330.14	43.84	3.78	23.93	4.34	329.50
XXXI	*m*-Bromophenacyl	172–174 dec	AcOH–H_2O	$C_{12}H_{12}BrNO_5$	43.65	3.66	24.21	4.24	330.14	43.56	3.70	24.50	4.32	331.50
XXXII	*m*-Bromo-*p*-methoxyphenacyl	200–202 dec	DMF–H_2O	$C_{13}H_{14}BrNO_6$	43.35	3.92	22.19	3.89	360.17	43.20	3.90	22.06	3.99	361.50
XXXIII	β-Phenoxyethyl	152–153	EtOH	$C_{12}H_{15}NO_5$	56.91	5.97		5.53	253.25	56.77	5.87		5.58	252.00
XXXIV	β-(*p*-Fluorophenoxy)-ethyl	134–135	H_2O	$C_{12}H_{14}FNO_5$	53.14	5.20	7.00	5.17	271.25	53.31	5.30	7.27	5.04	272.00
XXXVI	β-(*p*-Bromophenoxy)-ethyl	173–174 dec	EtOH	$C_{12}H_{14}BrNO_5$	43.39	4.25	24.06	4.21	332.16	43.20	4.28	24.06	4.22	331.00
XXXVII	*p*-Bromoacetanilido	223–225 dec	EtOH	$C_{12}H_{13}BrN_2O_5$	41.76	3.79	23.15	8.11	345.16	41.65	3.86	22.95	8.16	348.00
XXXVIII	*p*-Iodoacetanilido	213–215 dec	DMF–H_2O	$C_{12}H_{13}IN_2O_5$	36.75	3.34	32.36	7.14	392.16	37.04	3.41	32.30	7.18	390.50

[a] Colorless needles. [b] Dimethylformamide.

followed by measuring the concentration of bromide ion (Volhard) in aliquots taken from the reaction mixture at different times. It was obtained as a colorless liquid, bp 133° (20 mm), lit.[18] bp 103–105° (21 mm).

Anal. Calcd for C_8H_8BrFO: C, 43.86; H, 3.68; Br, 36.48. Found: C, 44.08; H, 3.57; Br, 36.20.

2,4′-Dibromoacetanilide, for the synthesis of XVIII, was prepared in 90% yield by bromination of 2-bromoacetanilide in acetic acid at room temperature; mp 169–171° (EtOH). This compound has been prepared from the oxime of 4,ω-dibromoacetophenone through Beckmann rearrangement, and by acylation of *p*-bromoaniline with bromoacetyl chloride;[19] mp 169–170°.

2-Bromo-4′-iodoacetanilide (XX), for the synthesis of XIX, was prepared by the general method of Jacobs and Heidelberger[20] for the preparation of halogen-substituted acetanilides. It was obtained in 90% yield by the reaction of bromoacetyl bromide with *p*-iodoaniline in aqueous acetic acid in the presence of sodium acetate; mp 190–192° (EtOH).

Anal. Calcd for C_8H_7BrINO: C, 28.26; H, 2.08; N, 4.12. Found: C, 28.07; H, 2.04; N, 4.15.

N-(*p*-Iodobenzyl)-3,3′-iminodipropionic acid diethyl ester (XXII) was prepared in quantitative yield by treating *p*-iodobenzyl bromide (1 mole) with 3,3′-iminodipropionic acid diethyl ester[21] (2 moles) in dry benzene; bp 169° (0.05 mm), $n^{25.6}D$ 1.5328.

Anal. Calcd for $C_{17}H_{24}INO_4$: C, 47.12; H, 5.58; I, 29.29; N, 3.23; mol wt, 433.30. Found: C, 47.12; H, 5.54; I, 29.50; N, 3.39; mol wt, 434.50.

3,3′-Iminodipropionic acid diethyl ester, for the synthesis of XXII, was obtained in 90% yield by a two-stage method (a method reported in the literature[21] gave only 45% yield): (1) addition of benzylamine to ethyl acrylate giving N-benzyl-3,3′-iminodipropionic acid diethyl ester[22] (95% yield); (2) quantitative hydrogenolysis of the N-benzyl group in EtOH (10% Pd–C), yielding 3,3′-iminodipropionic acid diethyl ester. The hydrobromide salt of the imino ester melted at 52–53° (EtOH).

Anal. Calcd for $C_{10}H_{20}BrNO_4$: C, 40.28; H, 6.76; Br, 26.80; N, 4.70; mol wt, 298.19. Found: C, 40.10; H, 6.85; Br, 27.00; N, 4.65; mol wt, 297.50.

***p*-Xylene-α,α'-di(N,N′-3,5-diketopiperazine) (XXVII)** was prepared by heating N,N′-(*p*-xylene-α,α'-diimino)tetraacetic acid (XXVI) (0.01 mole), intimately mixed with urea (0.02 mole), to 170° (oil bath) until no more gas was evolved; yield 90%, mp 265–267° dec (dimethylformamide–H_2O).

XXVI

XXVII

Anal. Calcd for $C_{16}H_{18}N_4O_4$: C, 58.17; H, 5.49; N, 16.96. Found: C, 57.99; H, 5.48; N, 17.03.

β-(*p*-Fluorophenoxy)ethyliminodiacetic acid (XXXIV) was prepared in two ways: (a) by saponification of the imino methyl ester (XVI), and (b) by alkylation of β-(*p*-fluorophenoxy)-ethylamine (XXXV) with chloroacetic acid in aqueous alkaline solution. Compound XXXV was prepared by reduction of *p*-fluorophenoxyacetamide with $LiAlH_4$.

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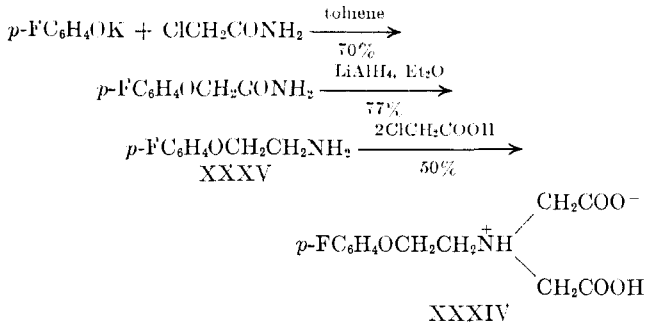

***p*-Fluorophenoxyacetamide**[23] was prepared in 70% yield by treating potassium *p*-fluorophenolate (1 mole) suspended in dry toluene with α-chloroacetamide (1 mole). The suspension was stirred and refluxed for 3 hr by which time the reaction was complete (phenolphthalein test, negative). The suspension was filtered hot and the filtrate was cooled in ice water to precipitate the product which was recrystallized from water; mp 111–112°. The Beilstein test for halogen was negative. Mixed with α-chloroacetamide it melted at 80–83° (depression); $\lambda_{max}^{CHCl_3}$ (μ) 2.84 (NH-amide stretching), 3.32 (aromatic CH), 5.90 (CO-amide), 6.30, 6.65 (aromatic C=C), 9.45 (C–F bond), 12.05 (*para*-substituted phenyl out of plane CH bending of 2 adjacent aromatic hydrogens).

Anal. Calcd for $C_8H_8FNO_2$: C, 56.80; H, 4.76; N, 8.28. Found: C, 57.05; H, 4.68; N, 8.35.

β-(*p*-Fluorophenoxy)ethylamine (XXXV) was prepared in 77% yield by reduction of the corresponding amide with $LiAlH_4$. Since the amide has a low solubility in ether it was placed in a Soxhlet thimble, and a Soxhlet apparatus was inserted between the flask containing the $LiAlH_4$ in dry ether and the reflux condenser. The reaction was complete after about 12 hr. After decomposing the excess $LiAlH_4$, the product was extracted from the ether with dilute H_2SO_4 (~2 *N*), and recovered from the aqueous solution by adding strong alkali and extracting with ether. The compound was obtained as a colorless distillate, bp 132.5° (20 mm), 63° (0.25 mm), $n^{28}D$ 1.5100.

Anal. Calcd for $C_8H_{10}FNO$: C, 61.92; H, 6.47; N, 9.03; mol wt, 155.18. Found: C, 62.20; H, 6.32; N, 8.92; mol wt, 156.00.

N-Benzyl-3,3′-iminodipropionic acid (XXXIX), colorless needles from 1-butanol, mp 147–148°.

Anal. Calcd for $C_{14}H_{17}NO_4$: C, 62.14; H, 6.82; N, 5.57; mol wt, 251.27. Found: C, 62.05; H, 7.02; N, 5.43; mol wt, 250.00.

N-(*p*-Iodobenzyl)-3,3′-iminodipropionic acid (XL), colorless needles, mp 153–155° (H_2O).

Anal. Calcd for $C_{13}H_{16}INO_4$: C, 41.40; H, 4.28; I, 33.65; N, 3.71; mol wt, 377.19. Found: C, 41.58; H, 4.42; I, 33.57; N, 3.72; mol wt, 375.50.

Attempted Saponification of C-(2-Pyridyl)nitrilotriacetic Acid Trimethyl Ester (XXI).—Saponification of this compound yielded N-(2-pyridylmethyl)iminodiacetic acid, mp 178–180° dec (equiv wt, 111.5; calcd equiv wt, 112.10), lit.[24] mp 174–175°, instead of the expected C-(2-pyridyl)nitrilotriacetic acid. Thus, it seems that the latter is very unstable and undergoes decarboxylation during the isolation process. This finding is supported by the fact that the amino acid 2-pyridylglycine is very unstable and easily decarboxylates to α-picolylamine.[25]

Preparation of Amino Acids Labeled with Radioactive Halogens. F^{18}-Labeled amino acids were prepared in two ways. (I) The first is recoil labeling of the organic compounds with "hot" extramolecular F^{18} produced in the nuclear reaction $Li^6(n,\alpha)H^3:O^{16}(H^3,n)F^{18}$. Because of the large cross section of the primary reaction $Li^6(n,\alpha)H^3$ with thermal neutrons (950 barns) substantial triton production is expected in neutron-irradiated samples of lithium salts of the amino acids. These tritons then react with the oxygen atoms in the molecules to yield F^{18}. The "hot" F^{18} atoms randomly substitute hydrogen atoms in the molecules.[26] (II) The second is direct production of F^{18}-labeled compounds from stable organic fluoro compounds by irradiation with fast neutrons in the reactor to produce the nuclear reaction $F^{19}(n,2n)F^{18}$. The yield in this reaction is, however, much smaller than in reaction I since in the fission-spectrum distribution, the abundance of neutrons which have an energy above the threshold of reaction II (10.4 mev) is rather small.

The activity of F^{18} (a positron emitter with a half-life of 112 min) is conveniently measured by its annihilation radiation (0.51 mev), in any conventional γ-counting equipment (well-type scintillation counter).

Procedure I.—Li^6 salts of amino acids were prepared by neutralizing their aqueous solutions with Li^6OH (triple-distilled water). The water was then evaporated and the Li^6 salts were dried to constant weight. The Li^6OH was prepared from Li^6 metal containing 96.1 atom % Li^6 obtained from Oak Ridge National Laboratory, Oak Ridge, Tenn. Samples of about 500 mg of the organic lithium salts were encapsulated in polyethylene vials. The vials were sealed and placed in the polyethylene carriers (rabbits) used in the pneumatic-tube facility of IRR-1 (the Israel 5MW swimming-pool reactor at Soreq). The rabbits were irradiated at the face of the reactor core where the thermal flux was 8×10^{12} neutrons/cm² sec, and the fast (fission spectrum) neutron flux was 1.8×10^{12} neutrons/cm² sec, at a cadmium ratio of 4.3. All of the irradiations were carried out with the reactor operating at a power level of 1.6 Mw. Samples were irradiated for 3 min, the irradiation dose being ~1.4×10^{15} nvt/cm². The irradiated salts were dissolved in NaF solution (~0.01 mole, as hold-back carrier for F^{18}), which was made alkaline with a few drops of NaOH. The solution was treated with active charcoal and heated for a few minutes, then cooled, filtered, and acidified in order to precipitate the free amino acids at their isoelectric points. Repetition of this procedure twice more yielded F^{18}-labeled amino acids with constant specific activity.

Procedure II.—F^{18}-labeled amino acids were produced directly by irradiating the stable fluoro amino acids under the same conditions as described for the lithium salts of the amino acids, after which they were treated as before.

Br^{82}-labeled amino acids were prepared in two ways. (I) The first was recoil labeling of amino acids with "hot" Br^{82} atoms produced in the $Br^{81}(n,\gamma)Br^{82}$ nuclear reaction. (II) The second was synthetic incorporation of Br^{82} into organic compounds. Br^{82} is a β and γ emitter (half-life 35.9 hr) and can be easily detected by its γ radiation in conventional γ-counting equipment.

Procedure I.—Recoil labeling of amino acids with "hot" Br^{82} atoms was carried out by mixing about 300 mg of the amino acid intimately with about 300 mg of ethylene bromide (BDH AnalaR) in a polyethylene vial, and irradiating the mixture under the conditions described before. After the irradiation, the samples were allowed to cool for approximately 48 hr in order to get rid of the Br^{80} activity ($t_{1/2}$ = 18 min) and to reduce the Br^{80m} activity ($t_{1/2}$ = 4.4 hr) to a minimum. The samples were then crystallized to constant specific activity from water containing NaBr (~0.1 mole) as a hold-back carrier for Br^{82}.

Procedure II.—Direct bromination with $Br^{82}{}_2$ was carried out with $Br^{82}{}_2$ obtained from irradiated NH_4Br. The NH_4Br samples (~500 mg) were irradiated in polyethylene vials in the reactor core for 20 min at 1.6 Mw. The samples were then allowed to cool for approximately 3 days, and $Br^{82}{}_2$ was generated according to the reaction $BrO_3^- + 5Br^- + 6H^+ \rightarrow 3Br_2 + 3H_2O$. The $Br^{82}{}_2$ was distilled carefully and collected in a small reservoir cooled with ice water. Before use, the $Br^{82}{}_2$ was dried by shaking with an equal volume of concentrated H_2SO_4. The total time required for preparing radioactive bromine and incorporating it into different organic compounds never exceeded 2 days.

I^{131}-labeled amino acids were prepared by isotopic exchange of stable iodo compounds with carrier-free I^{131}, a β and γ emitter ($t_{1/2}$ = 8.07 days). Since its principle γ radiation is of 0.364 mev, correction should be made for self-absorption when measuring its activity.

Procedure.—About 10 μmoles of the iodo amino acid was dissolved in 1 ml of methanol. To this solution, 0.1 ml of glacial acetic acid saturated with sodium bromate and a few microliters of carrier-free NaI^{131} solution (Radiochemical Centre, Amersham, England) was added. The solution was kept in a water bath at 40° for 2–3 days after which time a stable carrier, iodoanisic acid dissolved in methanol, was added, and the compound was precipitated by diluting the solution with water or acetone

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containing NaI (~0.1 mole) as a hold-back carrier for I^{131}. The I^{131}-labeled amino acid was redissolved in methanol and precipitated again by adding water or acetone containing stable NaI. In this way I^{131}-labeled amino acids were obtained with constant specific activity.

Radiochemical Purity Determinations. F^{18}-Labeled Compounds. I. γ-Spectrum Scan Using a γ Spectrometer, 3 × 3 in. NaI(Tl) Crystal, R.C.L. 512 Multichannel Pulse-Height Analyzer.—The spectrum was taken with an energy spread of approximately 2.5 mev to ensure that there was no interference from high-energy activities such as Cl^{38} ($t_{1/2}$ = 37.3 min, 1.67 mev, 2.19 mev) and Na^{24} ($t_{1/2}$ = 15.0 hr, 1.37 mev). Only one peak appeared in the γ spectrum (at 0.51 mev) corresponding to the annihilation radiation of the F^{18} positrons.

II. Decay Curves.—Activity measurements of the F^{18}-labeled compounds for about 6 hr after irradiation at intervals of 1 hr yielded a straight-line decay curve on semilogarithmic paper, with a half-life value of 105–120 min. Twenty-four hours after the irradiation no activity was detected.

Br^{82}-Labeled Compounds. I.—γ-Spectrum scan (0.55 mev, 0.61 mev, 0.76 mev).

II.—Decay curves (half-life = 36 ± 0.5 hr).

Paper Chromatography of Br^{82}- and I^{131}-Labeled Amino Acids.—Radiobromo- and radioiodo-labeled amino acids were subjected to ascending paper chromatography[15] to ensure the absence of free Br^{82} or I^{131} ions. The strip papers were then scanned in a 4π radiochromatogram scanner, Picker X-ray Corp. The R_f values obtained corresponded to those of the pure amino acids.

Quantification and Prediction of the Biological Activities of Chloramphenicol Analogs by Microbial Kinetics[1]

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The rate constants, k, for the growth of *Escherichia coli* are linearly related to the concentrations of the chloramphenicol analog, [I], *i.e.*, $k = k_0 - k_I[I]$ where the inhibitory constant k_I gives a precise estimate of the substituent effect on biological activity by a total-count technique with the Coulter counter. The substituent effects for a series of ring-substituted chloramphenicol analogs of the D-*threo* configuration may be ranked p-NO_2 > p-SCH_3 > p-I > p-Br ~ m-NO_2 > p-OCH_3 > p-Cl > p-*i*-C_3H_7 > p-SO_2CH_3 > p-NH_2, where the p-SCH_3, p-SO_2CH_3, and m-NO_2 analogs are incongruent with the Hansch equation. The individual inhibitory constants, k_I, can be used to predict quantitatively the rate of *E. coli* growth in any admixture of analogs, *e.g.*, $k = k_0 - \Sigma_i k_i[I]_i$ and clearly demonstrate that, in this series, dose effects are additive with respect to rates.

The interest in correlating substituent constants and partition coefficients with biological activity as evidenced by the recent publications of Hansch and coworkers[2] has shown the vital need for the quantitative and precise evaluation of the biological activity of various substituted compounds. Only when such data are available can the models for such correlations be adequately tested and modified.

The complaint[2a] is legitimate that there is a severe limitation on the number of adequate examples in the literature where a particular compound has been modified with a wide variety of substituents and where these compounds have been tested under a standard set of conditions to yield quantitative results. In fact, an outstanding exception is considered[2a] to be a study of the action of chloramphenicol analogs on microorganisms by the serial-dilution method where the accuracy of the determinations was ±25%.[3] The need for quantification at a higher order of accuracy is readily apparent when parameters are to be correlated in the intermediate ranges of activities, *i.e.*, 25–100% of the most active analog.

It has been demonstrated that the total counts obtained with the use of the Coulter counter (Coulter Electronics, Hialeah, Fla.) of a growing *Escherichia coli* culture in the logarithmic growth phase in the presence of chloramphenicol and/or tetracycline are coincident with the viable counts by the colony-count technique[1c] and the mode of action is inhibitory. This provides a counting technique that could estimate growth rates rapidly and precisely (±2%) in the presence of various chloramphenicol analogs and permit the quantification of substituent effects on biological activity with confidence.

The apparent first-order generation rate constant can be determined from the slope of a plot of the logarithm of numbers, N, of *E. coli* inoculated in the constant growth phase since

$$N = N_0 e^{kt} \quad (1)$$

so that

$$\log N = \log N_0 + kt/2.303 \quad (2)$$

where t is in seconds. In the specific cases of chloramphenicol and tetracycline it has been shown[1] that the apparent first-order generation rate constant after the addition of antibiotic is linearly related to the antibiotic concentration

$$k = k_0 - k_I[I] \quad (3)$$

where k_0 is the rate constant in the absence of antibiotic and k_I is the specific inhibitory constant for the antibiotic I. These procedures permit the determination of various k_I values as more precise estimates of the effects of a compound's substituents on biological activity. Previous studies have shown that

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