Synthesis of α-Methylenebutyrolactams as Potential Antitumor Agents

MILTON J. KORNET

Received June 12, 1978, from the *Division of Medicinal Chemistry, College of Pharmacy, University of Kentucky, Lexington, KY 40506.*
Accepted for publication August 30, 1978.

Abstract □ A series of 1-aryl-3-methylene-2-pyrrolidinones was synthesized *via* a three-step reaction sequence. 1,4-Bis-[*N*-(3-methylene-2-oxopyrrolidino)]benzene, which can undergo alkylation at two sites, was also prepared. These compounds are related to the known antitumor agents α-methylenebutyrolactones. Attempts to prepare bis-α-methylenelactams, in which the heterocyclic rings are joined through their nitrogen atoms by an alkylene bridge, were unsuccessful. All of the α-methylenelactams were screened in B16 melanocarcinoma and P-388 lymphocytic leukemia tumor systems but failed to show significant activity.

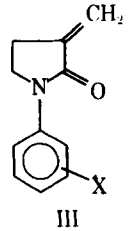
Keyphrases □ α-Methylenebutyrolactams—synthesized as potential antitumor agents and screened □ Pyrrolidinones, substituted—synthesized as potential antitumor agents, screened □ Antitumor agents, potential—synthesis and screening of α-methylenebutyrolactams

Investigations (1–3) on sesquiterpene lactones possessing cytotoxic and antitumor activities have shown that biological activity is largely dependent on the presence of an α-methylene-γ-lactone moiety. This activity has been ascribed to a rapid and essentially irreversible 1,4-addition reaction between the α,β-unsaturated lactone and the SH group of L-cysteine, either as the free amino acid or, more likely, as part of a protein molecule (4). Other biological nucleophiles may also be involved. Recent work has concentrated on the synthesis of simple α-methylenelactones (5–8) and has produced some active new compounds. Compounds that contain more than one alkylating group appear to enhance cytotoxicity significantly (5, 9).

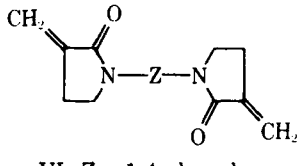
This report is concerned with the synthesis of mono- and bis-α-methylenelactam systems (III, VI, and IX) as potential antitumor agents. These investigations were prompted by the facts that an *N*-aryl-3-methylene-2-pyrrolidinone readily adds nucleophiles, such as cyanide ion and thiophenol (10), and that isosteric replacement of oxygen by nitrogen often leads to improved drugs. Moreover, there is a dearth of literature information on the antitumor properties of α-methylenelactams. Also noteworthy is the recent proposal (11) that the oncolytic actions of camptothecin may occur as a consequence of its bioreduction to an α-methylenevalerolactam intermediate, which then functions as an alkylating agent.

DISCUSSION

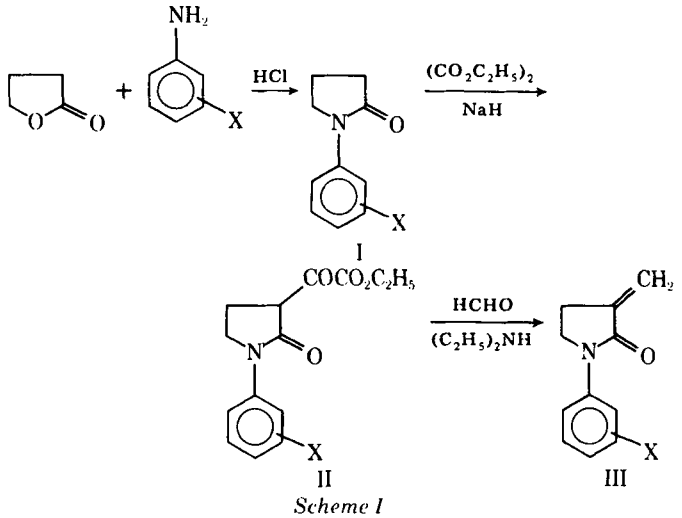
Chemistry—The synthesis of the mono-α-methylenelactam compounds (III) was achieved by a three-step reaction sequence similar to that used by Seidel and Cook (10) (Scheme I). Modifications of these



III



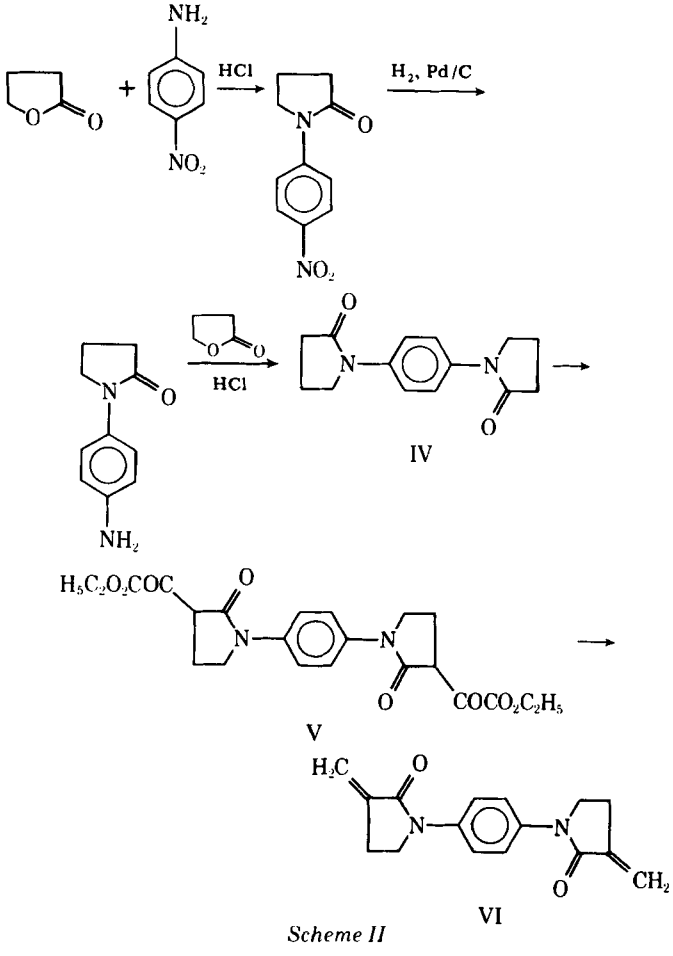
VI: Z = 1,4-phenylene
IX: Z = alkylene



Scheme I

procedures have been described (12). The physical properties of the intermediates and final products are listed in Tables I–III.

The last two steps used in the synthesis of the bis-α-methylenelactams



Scheme II

0022-3549/79/0300-0350$01.00/0

Table I—Physical Properties of 1-Aryl-2-pyrrolidinones (I)

Compound	X	Melting Point	Yield, %	Recrystallization Solvent[a]	Formula	Analysis, % Calc.	Found
I*a*	H	65.5–66.5°[b]	78	A	$C_{10}H_{11}NO$	— —	—
I*b*	*p*-Br	97.5–98.5°[c]	61	B	$C_{10}H_{10}BrNO$	— —	—
Ic	*o*-Cl	55–57°[d]	73	—	$C_{10}H_{10}ClNO$	— —	—
I*d*	*m*-Cl	64–65.5°[e]	85	C	$C_{10}H_{10}ClNO$	C 61.39	61.41
						H 5.15	5.24
						N 7.16	7.23
I*e*	*p*-F	54–55.5°	61	D	$C_{10}H_{10}FNO$	C 67.02	66.95
						H 5.63	5.62
						N 7.82	7.81
I*f*	*p*-OC_2H_5	111–113°	59[f]	B	$C_{12}H_{15}NO_2$	C 70.22	70.34
						H 7.37	7.38
						N 6.82	6.91
I*g*	*p*-NO_2	125.5–127°[g]	77[h]	E	$C_{10}H_{10}N_2O_3$	C 58.25	58.23
						H 4.89	4.85
						N 13.59	13.69
I*h*	*p*-CH_3	86–87.5°[i]	36	B	$C_{11}H_{13}NO$	— —	—
I*i*	*p*-NH_2	129–131°[j,k]	100	E	$C_{10}H_{12}N_2O$	C 68.16	68.19
						H 6.86	6.75
						N 15.90	15.86

[a] A = 35% aqueous ethanol, B = 50% aqueous ethanol, C = 70% aqueous ethanol, D = 25% aqueous ethanol, and E = 95% ethanol. [b] Lit. mp 65–66°; M. S. Manhas and S. J. Jeng, *J. Org. Chem.*, **32**, 1246 (1967). [c] Lit. mp 97–98°; see footnote *b* for reference. [d] Lit. mp 62–63°; S. S. Kukalenko and N. A. Gracheva, *Khim. Geterotsikl. Soedin.*, **1971**, 773. [e] Lit. mp 66–68°; D. Ludsteck and O. Kaufmann, German pat. 1,301,314 (1969); through *Chem. Abstr.*, **71**, 124226p (1969). [f] The corresponding phenol occurred as a by-product and was separated by washing the organic phase with 5% aqueous sodium carbonate solution. [g] Lit. mp 131°; W. Reppe *et al.*, *Justus Liebigs Ann. Chem.*, **596**, 158 (1955). [h] Reaction time of 48 hr. [i] Lit. mp 87.5°; J. T. Braunholtz and F. G. Mann, *J. Chem. Soc.*, **1957**, 4174. [j] Lit. mp 127°; see footnote *g* for reference. [k] Obtained by catalytic reduction of I*g*; see *Experimental*.

Table II—Physical Properties of 1-Aryl-3-ethoxalyl-2-pyrrolidinones (II)

Compound	X	Melting Point	Yield, %	Reflux Time, hr	Recrystallization Solvent[a]	Formula	Analysis, % Calc.	Found
II*a*	H	110–111°	76	3	A	$C_{14}H_{15}NO_4$	C 64.36	64.37
							H 5.79	5.81
							N 5.36	5.47
II*b*	*p*-Br	152.5–154°	80	7	B	$C_{14}H_{14}BrNO_2$	C 49.43	49.43
							H 4.15	4.22
							N 4.12	4.19
II*c*	*o*-Cl	123–125°	51	4	A	$C_{14}H_{14}ClNO_4$	C 56.86	56.81
							H 4.77	4.93
							N 4.74	4.87
II*d*	*m*-Cl	115–116°	50	3	A	$C_{14}H_{14}ClNO_4$	C 56.86	56.83
							H 4.77	4.86
							N 4.74	4.82
II*e*	*p*-F	121–122°	66	6	A	$C_{14}H_{14}FNO_4$	C 60.21	60.33
							H 5.05	5.16
							N 5.02	5.09
II*f*	*p*-OC_2H_5	126–130°	81	6.5	A	$C_{16}H_{19}NO_5$	C 62.94	62.97
							H 6.27	6.34
							N 4.59	4.61
II*g*	*p*-NO_2	185–186°	93	6	C	$C_{14}H_{14}N_2O_6$	C 54.90	55.00
							H 4.61	4.72
							N 9.15	9.16
II*h*	*p*-CH_3	127–128°	72	6	A	$C_{15}H_{17}NO_4$	C 65.44	65.56
							H 6.22	6.31
							N 5.09	5.19

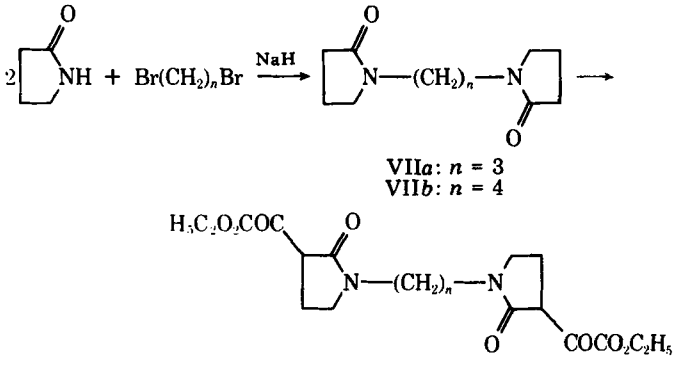
[a] A = 95% ethanol, B = benzene, and C = tetrahydrofuran.

(VI and IX) are identical to those used for the mono compounds (Scheme I). The reaction sequence used for the preparation of the precursor dilactam (IV) containing an arylene bridge is shown in Scheme II. The reaction employed for the synthesis of the precursor dilactams (VII) containing an alkylene bridge is shown in Scheme III.

All attempts to convert the bis-ethoxalyllactams (VIII*a* and VIII*b*) containing an alkylene bridge into the corresponding bis-α-methylenelactams gave intractable mixtures. In contrast, the conversion of the bis-ethoxalyllactam (V) containing a 1,4-phenylene bridge into the corresponding bis-α-methylenelactam (VI) succeeded.

Biological Results—Antineoplastic activity for III*a*–III*h* and VI was determined under the auspices of the National Cancer Institute, National Institutes of Health, according to general screening procedures (13). Both B16 melanocarcinoma and P-388 lymphocytic leukemia tumor systems were employed. Although initial assays of Ic, I*e*, and I*f* with P-388 leukemia indicated marginal activity (%T/C = 125–136), these results were not reproducible. The compounds also failed to show reproducible activity in the B16 melanocarcinoma assay. Because of insufficient material, VI was not assayed against B16 melanocarcinoma.

The apparent conclusion from these studies is that *N*-aryl nitrogen is not a beneficial replacement for the oxygen of α-methylenebutyrolactones. Further investigations are necessary to determine whether this result is due to steric or electronic factors in the reaction with biological



Scheme III

Table III—Physical Properties of 1-Aryl-3-methylene-2-pyrrolidinones (III)

Compound	X	Melting Point	Yield, %	Recrystallization Solvent[a]	Formula	Analysis, %	Calc.	Found
III*a*	H	77.5–78.5°	21	A	$C_{11}H_{11}NO$	C	76.28	76.44
						H	6.40	6.42
						N	8.09	8.17
III*b*	*p*-Br	125–126.5°	33	B	$C_{11}H_{10}BrNO$	C	52.40	52.36
						H	4.00	4.49
						N	5.56	5.67
III*c*	*o*-Cl	83–84°	32	A	$C_{11}H_{10}ClNO$	C	63.62	63.68
						H	4.85	4.86
						N	6.75	6.85
III*d*	*m*-Cl	78.5–80°	40	A	$C_{11}H_{10}ClNO$	C	63.62	63.71
						H	4.85	4.85
						N	6.75	6.90
III*e*	*p*-F	90–91.5°	29	A	$C_{11}H_{10}FNO$	C	69.09	69.13
						H	5.27	5.35
						N	7.33	7.40
III*f*	*p*-OC_2H_5	89–90.5°	23	A	$C_{13}H_{15}NO_2$	C	71.87	71.82
						H	6.96	7.01
						N	6.45	6.47
III*g*	*p*-NO_2	175–176°	41	C	$C_{11}H_{10}N_2O_3$	C	60.55	60.64
						H	4.62	4.76
						N	12.84	12.88
III*h*	*p*-CH_3	92–93°	32	A	$C_{12}H_{13}NO$	C	76.98	77.09
						H	7.00	7.11
						N	7.48	7.52

[a] A = cyclohexane, B = benzene–cyclohexane, and C = isopropyl alcohol.

nucleophiles or to poor transport of the compounds across cell membranes.

EXPERIMENTAL[1]

1-Aryl-2-pyrrolidinones (I)—A typical reaction is described: that for the preparation of 1-(*m*-chlorophenyl)-2-pyrrolidinone (I*d*) (Table I). A mixture of 34.4 g (0.40 mole) of butyrolactone, 56.1 g (0.44 mole) of *m*-chloroaniline, and 10.7 ml of concentrated hydrochloric acid was heated under a water-cooled reflux condenser for 2.5 hr in an oil bath at 150°. After changing to an air-cooled condenser, heating was continued at 180–190° for 17 hr. The reaction mixture was cooled and treated with 100 ml of 2 *N* HCl. The resulting tan solid was filtered and recrystallized to afford 66.5 g of off-white crystals.

1-Aryl-3-ethoxalyl-2-pyrrolidinones (II)—A typical reaction is described: that for the preparation of 1-(*p*-ethoxyphenyl)-3-ethoxalyl-2-pyrrolidinone (II*f*) (Table II). To a stirred, refluxing mixture of 8.43 g (0.20 mole) of a 57% mineral oil dispersion of sodium hydride in 100 ml of dry tetrahydrofuran, protected by a nitrogen atmosphere, was added dropwise a solution of 20.5 g (0.10 mole) of 1-(*m*-chlorophenyl)-2-pyrrolidinone and 29.2 g (0.20 mole) of diethyl oxalate in 300 ml of dry tetrahydrofuran.

Hydrogen gas was evolved during the addition as well as during the subsequent reflux period of 6.5 hr. The reaction mixture was then cooled and treated dropwise with 12 ml of acetic acid. After stirring for 10 min, the mixture was poured into 1.5 liters of ice water, and the precipitated solid was filtered and dried. The dry solid was triturated with petroleum ether to remove mineral oil. Recrystallization from 95% ethanol afforded 24.5 g of product.

1-Aryl-3-methylene-2-pyrrolidinones (III)—A typical reaction is described: that for the preparation of 1-(*p*-tolyl)-3-methylene-2-pyrrolidinone (III*h*) (Table III). To a magnetically stirred mixture of 21.8 g (0.079 mole) of 1-(*p*-tolyl)-3-ethoxalyl-2-pyrrolidinone, 26.5 ml of 48% aqueous diethylamine, and 135 ml of distilled water was added dropwise 26.5 ml of 40% aqueous formaldehyde while the temperature was maintained at 25–30°. After stirring at room temperature for 22.5 hr, the mixture was acidified with 10% HCl to pH ~ 4 and extracted three times with 100-ml portions of chloroform.

The combined extracts were dried (magnesium sulfate), filtered, and concentrated. The oily residue (16.9 g) was chromatographed on 130 g of silica gel 60. Elution with benzene and benzene–ethyl acetate solutions of increasing polarity gave 4.79 g of white solid; IR (potassium bromide): 5.93 μm (C=O); NMR ($CDCl_3$): δ 2.30 (s, 3, CH_3), 2.50–3.10 (m, 2, NCH_2CH_2), 3.73 (t, 2, NCH_2CH_2), 5.28–5.50 (m, 1, $C{=}CH_2$), 5.98–6.22 (m, 1, $C{=}CH_2$), 7.15 (d, 2, ArH), and 7.62 (d, 2, ArH) ppm.

1-(*p*-Aminophenyl)-2-pyrrolidinone (I*i*)—A suspension of 10.3 g (0.05 mole) of 1-(*p*-nitrophenyl)-2-pyrrolidinone (II*g*) and 0.6 g of 5% palladium-on-charcoal in 150 ml of 95% ethanol was shaken on the Parr hydrogenator for 1.25 hr. The reaction mixture was filtered, and the filtrate was evaporated under reduced prssure. The remaining white solid amounted to a quantitative yield, mp 124–126°. Recrystallization from 95% ethanol gave an analytically pure product.

1,4-Bis-[*N*-(2-oxopyrrolidino)]benzene (IV)—This compound was obtained from 8.8 g (0.05 mole) of I*i*, 6.45 g (0.075 mole) of butyrolactone, and 2.5 ml of concentrated hydrochloric acid by the identical procedure used for the preparation of I. Workup and recrystallization from 95% ethanol afforded 5.72 g (47%) of crystals, mp 251–253°.

Anal.—Calc. for $C_{14}H_{16}N_2O_2$: C, 68.83; H, 6.60; N, 11.47. Found: C, 68.91; H, 6.60; N, 11.47.

1,4-Bis-[*N*-(3-ethoxalyl-2-oxopyrrolidino)]benzene (V)—This compound was obtained from 4.47 g (0.018 mole) of IV, 10.5 g (0.072 mole) of ethyl oxalate, and 3.0 g (0.072 mole) of a 57% mineral oil dispersion of sodium hydride according to the same procedure used for the preparation of II. Workup gave 7.65 g (96%) of solid, mp 260–264°. Recrystallization from dimethylformamide gave an analytically pure product, mp 276–278°.

Anal.—Calc. for $C_{22}H_{24}N_2O_8$: C, 59.46; H, 5.44; N, 6.30. Found: C, 59.80; H, 5.13; N, 6.27.

1,4-Bis-[*N*-(3-methylene-2-oxopyrrolidino)]benzene (VI)—This compound was obtained from 6.51 g (0.0147 mole) of V, 9.87 ml of 48% aqueous diethylamine, 9.87 ml of 40% aqueous formaldehyde, and 60 ml of distilled water according to the procedure used for the preparation of III. Workup gave 2.9 g of crude product. Purification was effected by preparative TLC on silica gel[2], employing multiple development (10 developments were required) with benzene–ethyl acetate (4:1). The progress of the purification was followed visually at 254 nm.

Chromatography of 1.5 g of the crude product on six 20 × 20-cm glass plates, followed by extraction of the product band with chloroform–methanol (1:1), afforded 312 mg of solid; this material moved as one spot in several solvent systems. Recrystallization from methanol gave an analytically pure material, mp 475° dec. (sealed tube); IR (KBr): 5.93 μm (C=O); NMR (trifluoroacetic acid): δ 2.35–2.93 (m, 4, NCH_2CH_2), 3.42–3.93 (m, 4, NCH_2CH_2), 5.23–5.53 (m, 2, $C{=}CH_2$), 5.73–6.01 (m, 2, $C{=}CH_2$), and 7.26 (s, 4, ArH) ppm; mass spectrum: *m/e* 268 (M^+).

Anal.—Calc. for $C_{16}H_{16}N_2O_2$: C, 71.62; H, 6.01; N, 10.44. Found: C, 71.61; H, 5.95; N, 10.45.

1,4-Bis-[*N*-(2-oxopyrrolidino)]butane (VII*b*)—Sodium hydride (57% mineral oil dispersion), 27.36 g (0.65 mole), was rapidly weighed, washed three times with hexane and twice with xylene, suspended in 400

[1] Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. IR spectra were taken on a Perkin-Elmer 700 spectrophotometer as liquid films or potassium bromide pellets. NMR spectra were recorded on a Varian A-60A spectrometer with tetramethylsilane as the internal reference. Elemental analyses were performed by Dr. Kurt Eder, Geneva, Switzerland.

[2] Merck F-254 precoated TLC plates, 2-mm thick layer.

ml of xylene, and charged into a reaction flask. To this stirred mixture, 51.06 g (0.60 mole) of 2-pyrrolidinone in 125 ml of xylene was added dropwise at 60–80°. The initial vigorous evolution of hydrogen abated after 1 hr. The mixture was heated to 140–150°, and 53.98 g (0.25 mole) of 1,4-dibromobutane in 100 ml of xylene was added dropwise.

The mixture was then heated to reflux and refluxed for 17 hr. After cooling to 60°, 8.6 ml of acetic acid was added, and stirring was continued for 10 min. The mixture was filtered using glass filter paper, and the solids were washed with 150 ml of benzene. The filtrate was concentrated under reduced pressure (water pump). Vacuum distillation (bath temperature of 140–190°, 0.4 mm Hg) removed unreacted starting materials. Upon cooling, the product solidified in the distillation flask. Recrystallization from benzene–cyclohexane afforded 35.1 g (63%) of product, mp 69–77°. A second recrystallization gave analytically pure material, mp 82.5–85°; IR (potassium bromide): 6.0 μm (C=O).

Anal.—Calc. for $C_{12}H_{20}N_2O_2$: C, 64.26; H, 8.99; N, 12.49. Found: C, 64.28; H, 8.91; N, 12.61.

1,3-Bis-[*N*-(2-oxopyrrolidino)]propane (VII*a*)—This compound was obtained from 21.3 g (0.25 mole) of 2-pyrrolidinone, 20.2 g (0.10 mole) of trimethylene bromide, and 16.8 g (0.40 mole) of a 57% mineral oil dispersion of sodium hydride according to the procedure for VII*b*. Workup and distillation gave 16.5 g (79%) of liquid, bp 177°/0.36 mm; IR (film): 5.97 μm (C=O).

Anal.—Calc. for $C_{11}H_{18}N_2O_2$: C, 62.83; H, 8.63; N, 13.32. Found: C, 62.95; H, 8.72; N, 13.28.

1,3-Bis-[*N*-(3-ethoxalyl-2-oxopyrrolidino)]propane (VIII*a*)—A suspension of 16.8 g (0.40 mole) of a 57% mineral oil dispersion of sodium hydride in 140 ml of dry tetrahydrofuran was heated to reflux. To it was added a solution of 58.5 g (0.40 mole) of diethyl oxalate and 21.0 g (0.10 mole) of VII*a* in 260 ml of dry tetrahydrofuran dropwise with stirring. Hydrogen evolution was monitored with a mercury bubbler.

After completion of the addition, the mixture was refluxed for 15 hr. After cooling to room temperature, 23 ml of acetic acid was added, and stirring was continued for 15 min. The mixture was poured into 1500 ml of ice water with stirring, and the precipitate was filtered and dried in a vacuum desiccator. Trituration with 50 ml of petroleum ether (bp 30–60°) and filtration gave 19.8 g (48%) of product, mp 121–123°. Recrystallization from 95% ethanol led to the pure compound, mp 124.5–126°.

Anal.—Calc. for $C_{19}H_{26}N_2O_8$: C, 55.60; H, 6.39; N, 6.83. Found: C, 55.63; H, 6.42; N, 6.58.

1,4-Bis-[*N*-(3-ethoxalyl-2-oxopyrrolidino)]butane (VIII*b*)—This compound was obtained from 11.2 g (0.05 mole) of VII*b*, 29.2 g (0.20 mole) of diethyl oxalate, and 8.42 g (0.20 mole) of a 57% mineral oil dispersion of sodium hydride according to the procedure for VIII*a*. Workup gave 13.0 g (61%) of solid, mp 142–147°. Recrystallization produced an analytically pure product, mp 146–148°.

Anal.—Calc. for $C_{20}H_{28}N_2O_8$: C, 56.60; H, 6.65; N, 6.60. Found: C, 56.42; H, 7.14; N, 6.69.

Pharmacological Testing—*P-388 Assay*—Ascitic fluid containing about 6×10^6 cells was inoculated intraperitoneally into male CDF_1 mice. In this assay, median survival times of %T/C ≥ 125 are considered significant. The compounds were administered by the intraperitoneal route in a saline plus alcohol vehicle. Nine daily doses were given, starting 24 hr after tumor inoculation.

B16 Melanocarcinoma Assay—B16 melanocarcinoma homogenate (dilution 1:10) was inoculated intraperitoneally into male BDF_1 mice. In this assay, median survival times of %T/C ≥ 125 are considered significant. Administration and dosing of compounds were the same as for the P-388 testing.

REFERENCES

(1) K.-H. Lee, E.-S. Huang, C. Piantadosi, J. S. Pagano, and T. A. Geissman, *Cancer Res.*, **31**, 1649 (1971).

(2) S. M. Kupchan, M. A. Eakin, and A. M. Thomas, *J. Med. Chem.*, **14**, 1147 (1971).

(3) P. A. Grieco, J. A. Noguez, Y. Masaki, K. Hiroi, M. Nishizawa, A. Rosowsky, S. Oppenheim, and H. Lazarus, *ibid.*, **20**, 71 (1977).

(4) S. M. Kupchan, D. C. Fessler, M. A. Eakin, and T. J. Giacobbe, *Science*, **168**, 376 (1970).

(5) A. Rosowsky, N. Papathanasopoulos, H. Lazarus, G. E. Foley, and E. J. Modest, *J. Med. Chem.*, **17**, 672 (1974).

(6) K.-H. Lee, T. Ibuka, S.-H. Kim, B. R. Vestal, I. H. Hall, and E.-S. Huang, *ibid.*, **18**, 812 (1975).

(7) P. A. Grieco, J. A. Noguez, Y. Masaki, K. Hiroi, M. Nishizawa, A. Rosowsky, S. Oppenheim, and H. Lazarus, *ibid.*, **20**, 71 (1977).

(8) I. K. Stamos, S. Evans, A. McKenzie, S. R. Byrn, and J. M. Cassady, Abstracts of 174th ACS Meeting, Medi 56, Chicago, Ill., 1977.

(9) K.-H. Lee, S.-H. Kim, C. Piantadosi, E.-S. Huang, and T. A. Geissman, *J. Pharm. Sci.*, **63**, 1162 (1974).

(10) M. C. Seidel and R. S. Cook, *J. Heterocycl. Chem.*, **3**, 311 (1966).

(11) H. W. Moore, *Science*, **197**, 527 (1977).

(12) M. J. Kornet, A. M. Crider, and E. O. Magarian, *J. Med. Chem.*, **20**, 1210 (1977).

(13) R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, **3** (2), 1 (1972).

ACKNOWLEDGMENTS

The author is indebted to Mr. Marshall Davis, Mr. George Piltz, and Mr. James Swencki for the syntheses of some of the compounds.

Facile *In Vitro* Method for Screening Inhibitors of IgE Binding to Mast Cells

STEPHEN M. COUTTS ˣ, DOROTHY M. REID, and IRA WEINRYB

Received July 6, 1978, from the *Department of Biochemistry and Drug Disposition, USV Pharmaceutical Corporation, Tuckahoe, NY 10707.* Accepted for publication August 28, 1978.

Abstract □ A method for rapidly testing large numbers of chemical structures as potential modulators of the interaction between immunoglobulin E (IgE) and its specific receptors on rat peritoneal mast cells is described. IgE, isolated from the ascitic fluid of a transplantable rat IgE immunocytoma, is labeled with iodine-125 under mild conditions employing the Bolton–Hunter reagent. The antibody is incubated with mixed peritoneal cells at 37°, and the cell-bound IgE is separated from unbound label by sedimentation through an 8% sucrose–polymer solution in microsediment tubes. Optimal conditions for the interaction of 3 n*M* IgE with 3×10^5 mast cells in 150 μl are: incubation time, 2 hr; pH, 6.5–7.0; and ionic strength, equivalent to 150 m*M* NaCl. Mixed peritoneal cells bind IgE with an affinity equal to that of purified mast cells. Human IgE pentapeptide III and several antiallergic agents do not compete with rat IgE in this assay.

Keyphrases □ Immunoglobulin E—binding to mast cells, *in vitro* method for screening inhibitors □ Binding—immunoglobulin E to mast cells, *in vitro* method for screening inhibitors □ Inhibitors—immunoglobulin E to rat peritoneal mast cells, *in vitro* screening method

The immunologically induced secretion of mediators of anaphylaxis by sensitized rat or human mast cells exposed to the appropriate antigen triggers a series of intracellular events that require Ca^{2+}, involve an activatable

0022-3549/79/0300-0353$01.00/0