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Structure-Activity Relationship of Some Diamine Bis-epoxides in Mouse Leukaemia*

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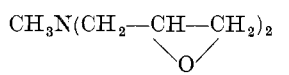
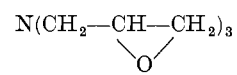
An extensive study of the relationship between the structure of a number of bis-epoxides and their activity against the Walker carcinosarcoma 256 in the rat has been reported by Ross.[1] This study revealed a distinct correlation of the chemical reactivity of the epoxide function towards anions and the observed biological effectiveness. Diepoxybutane was found to be one of the most active compounds, while a few primary aromatic amine bis-epoxides, e.g. N,N-bis(2,3-epoxypropyl)anisidine, were reported to possess low activity against the tumour system employed.

In our laboratories, using the mouse leukaemia P1534 as the biological assay system,† two related epoxides, N,N-bis(2,3-epoxy-*n*-propyl)methylamine (Ia) and tris(2,3-epoxy-*n*-propyl)-amine (Ib), likewise showed only slight (Ia) or negligible (Ib)



Ia



Ib

activity in extending the survival time of treated mice over that of controls. When a high degree of activity was observed, how-

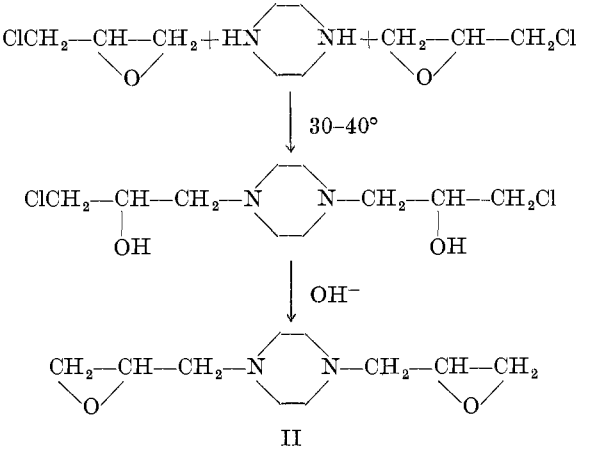
* Presented before the Medicinal Chemistry Section in the American Chemical Society Meeting, Chicago, Illinois, September, 1958.

† The biological part of this work was performed by Dr. I. S. Johnson and Mr. Howard F. Wright of our Biological Research Division. For a general description of techniques reference is made to C. C. Stock, Aspects of Approaches in Experimental Cancer Chemotherapy, *Amer. J. Med.*, **8**, 658 (1950). Certain aspects of this phase of the work were presented at the 7th International Cancer Congress, July 9, 1958, London, and are to be published in the *ACTA* of the International Union against Cancer.

ever, in a di-secondary amine derivative, namely, N,N′-bis-(2,3-epoxy-*n*-propyl)piperazine (II), it became of interest to study the effect of structural modifications, particularly of the amine function, on the anti-leukaemic activity.

Chemistry of Bis-epoxides (I–XII)

The preparation* of the bis-epoxides, listed in Table I, involved the initial condensation of the purified amines with 2 moles of freshly distilled epichlorohydrin (β-methylepichlorohydrin for IV) in ethanol or methanol solution at 30–40°. Treatment with concentrated alkali converted the bis-chlorohydrins thus formed to the bis-epoxides which were purified by distillation under reduced pressure and/or crystallization from anhydrous ether at −50°. Distillation of the crude products often led to violent decomposition and total loss of material but purified bis-epoxides were distilled safely.



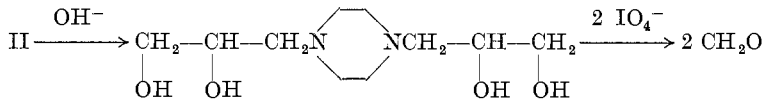
II

The epoxide values obtained by the Ross assay† indicate the correctness of the proposed structure. To confirm structure II

* The reaction of mono-secondary amines and epichlorohydrin has recently been reviewed: *Epichlorohydrin, Technical Booklet SC: 49–35*, Shell Chemical Corporation, 1949.

† The epoxide assay using sodium thiosulphate described by Ross has been modified and adapted for use with the basic epoxides reported here. Details of this method will be published elsewhere.

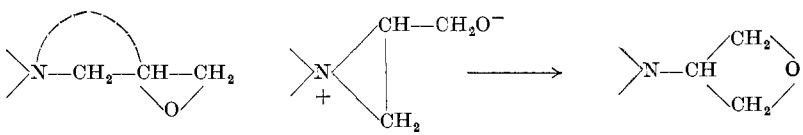
—and in so doing rule out the alternate possibility of a 2-substituted 1,3-epoxypropyl side chain*—the bis-epoxide was hydrolysed with dilute base and the resulting N,N′-bis(2,3-dihydroxy-propyl)piperazine¶ oxidized *in situ* with 2 moles of sodium metaperiodate. The yield of formaldehyde thus obtained was about 85 per cent of theory.



The solid bis-epoxides (II through VII) are fairly stable at 0° but require quarterly or yearly re-crystallization to remove polymeric material. The liquid products (Ia, Ib, IX through XII) are less stable and for testing purposes need to be re-distilled weekly or monthly. All products with the exception of VII are sufficiently soluble in water to allow the use of this solvent for parenteral administration.

Though all but a few of the bis-epoxides are of satisfactory purity as judged by the usual criteria, the epoxide content is

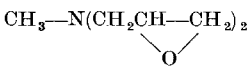
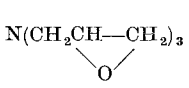
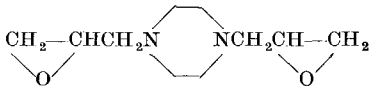
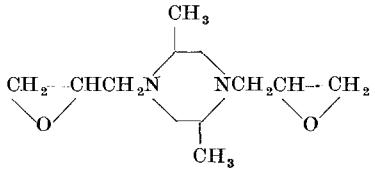
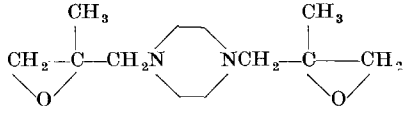
* Substitution by piperazine at the *centre* carbon of epichlorohydrin would yield a 1,3-chlorohydrin which upon treatment with alkali would give the 3-substituted trimethylene oxide *directly*. Theoretically, it would also seem possible that the trimethylene oxide ring is formed *indirectly* by way of the 2,3-epoxypropyl function; somewhat analogous to rearrangements observed with β-chloroethylamines (S. D. Ross, *J. Amer. chem. Soc.*, **69**, 2982 (1947) the initially formed epoxide, upon further reaction, could form an intermediate ethylenimonium alkoxide yielding the trimethylene oxide derivative as the final product.

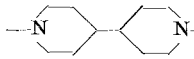
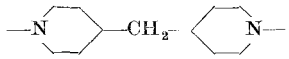
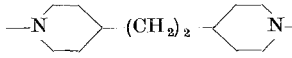
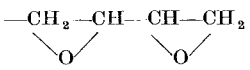


This indirect route is rendered unlikely in view of the normal ring opening with alkali reported here for II.

¶ Titration with sodium metaperiodate at pH 5–6 of a synthetic sample of this tetrol (m.p. 180°C), prepared from piperazine and 2 moles of glycidol (see experimental section) revealed an instantaneous uptake of 2 moles, followed by the rapid consumption of 2 additional, 'abnormal' moles of this reagent. Other instances of 'abnormal' oxidations with this reagent have been reported: E. H. Flynn, M. V. Sigal, Jr., P. F. Wiley and K. Gerzon, *J. Amer. chem. Soc.*, **76**, 3124, 3130 (1954); K. Gerzon, E. H. Flynn, M. V. Sigal, Jr., P. F. Wiley, R. Monahan and U. C. Quarck, *J. Amer. chem. Soc.*, **78**, 6396 (1956).

Table I

No.	Structure	Epoxide %	pK′$_a$ values*	Activity/ max. dose	Rel. biol. effectiveness†
Ia		95	<3·4 (9)	0–10/3	0·1
Ib		94	4·4	0/3	0
II		96–97	<3; 6·1 (5·9; 9·4)	120/30	1
II:	'*meso*-isomer'	88	<3; 6·2	80/30	0·7
III		100	<3; 6·4 (5·2; 9·4)	125/150	0·2
IV	*trans* 	100	<3; 6·1	0/120	0

	R				
V		91–92	6·8; 7·9 (9·5; 10·7)	170/3	5–10
VI		85–86	7·0; 8·0 (9·9; 10·5)	135/1·5	10–20
VII		89–90	7·1; 8·1 (9·9; 10·6)	0–10/6	0·1
VIII	—(CH$_3$)N—(CH$_2$)$_2$—N(CH$_3$)—§	< 30	— (6·6; 9·9)		
IX	—(CH$_3$)N—(CH$_2$)$_3$—N(CH$_3$)—	83	5·4; 7·8 (8·3; 10·4)	‡	
X	—(CH$_3$)N—(CH$_2$)$_4$—N(CH$_3$)—	96·5	6·4; 8·0 (8·9; 10·6)	105/6	3
XI	—(CH$_3$)N—(CH$_2$)$_6$—N(CH$_3$)—	89	7·1; 7·8 (9·5; 10·6)	105/3	5–10
XII	—(CH$_3$)NCH$_2$—C≡C—CH$_2$N(CH$_3$)—	94	4·0; 5·6 (6·7; 8·6)	‡	0·1(?)
XIII		95	—	0–10/3	0·1

* Measured in 66 per cent dimethylformamide. The second pair of figures (bracketed) represents the pK′a values for the intermediate amines.
† See text.
‡ No satisfactory test obtained.
§ Compound does not have the expected structure; see text.

usually found to be low, viz. 96–97 per cent of theory for II, 91–92 per cent for V, and 85–86 per cent for VI. It has not been possible to ascertain whether this discrepancy is due to an inherent shortcoming of the thiosulphate assay when applied to the present basic group of bis-epoxides or perhaps to the presence of isomeric byproducts. Relatively small amounts of such byproducts containing a trimethylene oxide or—less likely—a dioxan ring* could well escape detection.

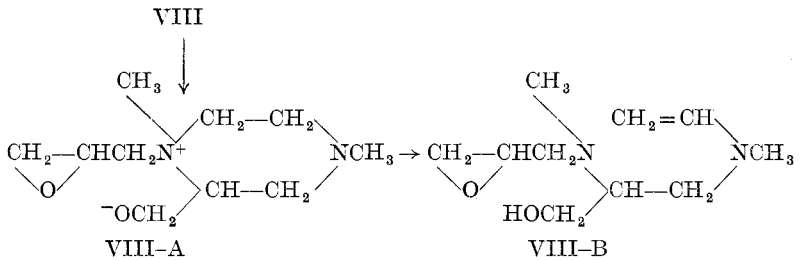
Occasionally, in the course of the preparation of the piperazine bis-epoxide (II), m.p. 42–43°, on a larger scale a less ether-soluble, higher melting isomeric bis-epoxide (IIi), m.p. 63°, was isolated in yields of about 5–10 per cent. Inasmuch as all physical characteristics (infra-red spectra, electrometric titration curves), except those associated with the solid state, were very similar, and as no interconversion has been observed when the lower melting isomer was crystallized in the presence of the higher melting one, it is believed that II and IIi represent the *racemic* and *meso*-form, respectively, of this species. This view is supported by the observation that the toxicity and biological effectiveness of II and IIi are of the same order.

The number of compounds reported here is small and the useful agents (II, V) are few indeed. The anti-leukaemic assay by its very nature and because of the time required renders impractical the quantitative comparison of a large number of compounds synthesized over a period of time. However, the major factor restricting the number of bis-epoxides in this series stems from the limitations imposed by their chemical and physical nature. Thus, N,N′-bis(2,3-epoxypropyl)-N,N′-dimethyltrimethylenediamine (IX) was twice lost on distillation; a third satisfactory preparation failed to provide information due to an assay failure.

Furthermore, an effort to prepare the bis-epoxide from N,N′-dimethylethylenediamine (VIII) led to unexpected results. Attempted distillation of the crude product resulted in a vigorous exothermic decomposition. Without the further application of external heat the distillation then proceeded to completion to give a clear, mobile, pale-yellow distillate which polymerized in a

* For a pertinent discussion of the factors influencing the course of this reaction including the conditions leading to the formation of dioxane derivatives, see: D. L. Heywood and B. Phillips, *J. Amer. chem. Soc.*, **80**, 1257 (1958).

matter of hours to a highly viscous product. Analysis of the fresh distillate revealed the expected composition of VIII, but the epoxide content was only 10–30 per cent of theory and the infrared spectrum showed a prominent absorption maximum at 6·05 μ. A plausible explanation of these events involves the initial formation of the quaternary alkoxide (VIII–A) which may be expected



to undergo a further Hofmann-type degradation yielding a vinylamine* such as VIII–B.

Irrespective of the details of this reaction scheme, it seems reasonable to expect that bis-epoxides will be unstable, as is VIII, when capable of forming a 6-membered ring (as in VIII–A) or a 5-membered ring. Thus, a bis-epoxide derived, for example, from *sym*-dimethylhydrazine should be unstable and initial plans for its preparation were therefore abandoned. Similarly, there is little incentive to study N-3,4-epoxybutyl analogues even if feasible synthetic methods were available. As expected, the bis-epoxides derived from tetramethylene- (X) and hexamethylenediamine (XI), which do not provide for such intramolecular interaction, were prepared without difficulty. That the piperazine bis-epoxide (II) does not suffer from the same instability observed in the closely related ethylene-diamine derivative (VIII) must be due to the greater rigidity of the piperazine ring.

Biological Activity

With respect to biological activity, a scrutiny of structure, basicity, and relative anti-leukaemic effectiveness† of the bis-

* A discussion of infrared spectra of enamines is given by N. J. Leonard and V. W. Gash, *J. Amer. chem. Soc.*, **76**, 2781 (1954).

† Treatment of a group of leukaemic mice consisted of 10 daily intraperitoneal injections of a freshly prepared aqueous solution of the drug. The mean survival

epoxides listed in Table I reveals two correlations. First, as noted previously,[1] a steric factor is noticeable: methyl substitution in the piperazine ring (III) or in the side chain (IV), while not appreciably affecting the basicity, either diminishes or abolishes the activity of the parent compound (II). Secondly, an increase in basicity leads, by and large, to considerably increased effectiveness in the open chain compounds (X and XI) and the piperidine analogues (V and VI). An exception is the 4,4′-bipiperidylethane bis-epoxide (VII); possibly, the realization of its potential activity is hindered by low solubility in water.

Without attempting to speculate on the actual physical or physiological basis for the observed increase of effectiveness* with basicity,† it is clear that in a physiological medium the more basic species will exist to a major degree in the ionized (protonated) form. This will lead, as Ross has demonstrated, to greatly increased reactivity of the epoxide group towards anions.‡ Also, it is recognized that increased basicity, besides its effect on chemical reactivity, may alter tissue distribution of a drug *in vivo*. Whether one or the other factor or, as is more probable, a combination of these and still other factors furnishes the basis for the observed increase in biological activity cannot be settled here. A statement of Ross sums up an important facet of this issue and applies probably even more accurately to the present group of basic bis-epoxides: 'This type of compound would be expected to react more readily with nucleophilic centres in regions where acidity is relatively higher. This might be significant from the standpoint of selective action towards different tissues.'

time of this group was determined and compared with that of saline treated infected controls (usually about 14 days).

The relative anti-leukaemic effectiveness of each drug was arrived at by dividing the mean *extension* of survival time by the maximum tolerated dose (mg/kg) producing this effect (Table I, column 4) and then expressing these values for the effectiveness (specific activity) in terms of that of II at unity (column 5).

* 'Effectiveness' is used here as defined above as specific activity. Due to the complexities inherent in the leukaemic assay, it has not been found feasible to establish the more significant Therapeutic Index for the bis-epoxides studied here and the use of this term has therefore been avoided in the text.

† A discussion of the relation of basicity and chemotherapeutic action is presented by A. Albert, *Selective Toxicity*, p. 72, 1951. New York; Wiley.

‡ The formation of the methiodide of N,N-bis(2,3-epoxypropyl)-*p*-anisidine resulted in a more than tenfold increase of the rate constant of the reaction with thiosulphate ion.[1]

A suggestion concerning the importance of one of the factors mentioned above was gained from a comparison of the rate of the reaction of anions with the epoxy function of the piperazine (II) and 4,4′-bipiperidyl bis-epoxide (V) as well as of diepoxybutane (XIII).[1,2] Of three candidate anions, thiosulphate, hydroxide, and phosphate ion, the first two are of little direct biological interest; also, the course of hydrolysis could not be followed conveniently.* On the other hand, a study of phosphorolysis was considered attractive because of the biological significance attributed[3] to the interaction of epoxides and other alkylating agents with the phosphate groups of vital cell constituents, e.g. nucleic acids, possibly nucleotides.†

The course of phosphorolysis, using K_2HPO_4,[4] KH_2PO_4, and mixtures of these salts, can be followed readily by the disappearance of inorganic phosphate. Under the conditions of the experiment carried out in the physiological pH range (7–8) (see Fig. 1), diepoxybutane (XIII)‡[2] underwent phosphorolysis to the extent of 13 per cent in a 24 h period; about twice as much (27 per cent) phosphate ester was formed from the piperazine compound (II) under these conditions, while of the bipiperidyl bis-epoxide (V) over 60 per cent had reacted with phosphate ion. Thus, we observe a distinct parallel between increased relative biological effectiveness (Table I, column 5) of the three bis-epoxides (XIII, 0·1; II, 1; V, 10) and the increase of chemical reactivity towards phosphate ion. It seems plausible to conclude that *at a particular pH* greater basicity, because of more extensive protonation, leads to increased chemical reactivity towards phosphate ion and that this effect in turn may be responsible, at least in

* A study of the reaction with hydroxide ion is impractical because: (a) concurrent reaction with anions of the buffers used (acetate, phosphate) obscures the hydrolysis; (b) the non-stoichiometric course of the periodate titration (footnote ¶ page 225) precludes its use in determining the extent of glycol formation; and (c) acid base titration as used by Ross[1] here is complicated by the amine bases present.

† G. Hems [*Nature, Lond.* **181**, 1721 (1958)] has discussed evidence for the possible involvement of yet another site of action, namely the N_7-position of deoxyguanylic acid in deoxyribonucleic acid.

‡ In these experiments a commercial sample (Aldrich Chemical Company, Milwaukee, Wisconsin) of 1,2,3,4-diepoxybutane was used which is believed to consist predominantly of the *meso*-isomer ($n_D^{25} = 1{\cdot}4280$; epoxide content, 94·5 per cent). The nature of the sample used by Ross was not stated.

part, for the increased biological activity of the more basic species.*

It is noted in passing that the bipiperidylethane bis-epoxide (VII) which is of the same basicity as V, in spite of its lack of biological activity (see above), reacts with phosphate to the same extent (63 per cent) as does V.

The effect of a *change of pH* on the reactivity of the bis-epoxides XIII, II and V towards phosphate ion showed a striking difference between XIII on the one hand, and the basic species (II and V)

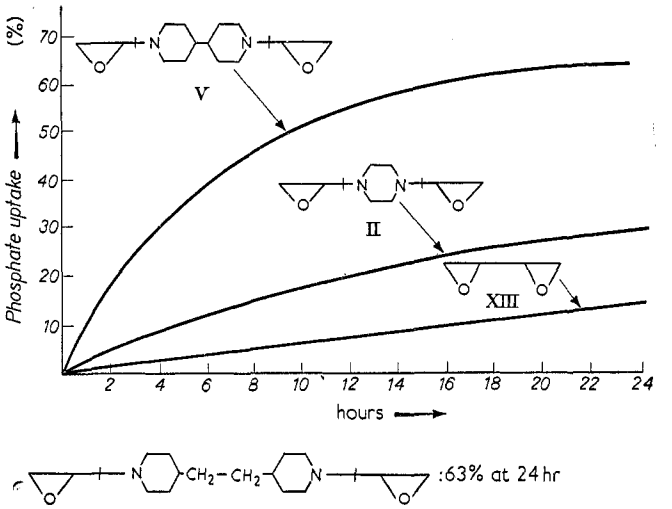


Fig. 1. Reaction with KH_2PO_4—K_2HPO_4 mixtures at pH 7–8.

on the other. The data (see Table III)show that a change towards *lower pH* values leads to *decreased* phosphorolysis with XIII, while both II and V undergo *increased* phosphorolysis. Evidently, the decrease in rate observed with XIII must be due mainly to an increasing proportion of the less reactive $H_2PO_4^-$ ion[4] at lower pH values, while for the basic epoxides (II and V) this rate lowering influence is more than reversed because of their greatly enhanced

* It is recognized that only one criterion, namely, the anti-leukaemic assay has been used here. Diepoxybutane reportedly has pronounced activity against the Walker carcinoma 256 in rats. We have not yet had the opportunity to compare this bis-epoxide with II and V against this tumour. See also J. Bichel, Treatment of Hodgkin's Disease with Diepoxybutane, *Abstracts of the 7th International Cancer Congress*, p. 32. July 1958. London.

reactivity at lower pH towards—the remaining fraction of—the HPO_4^{--} ion present. *Thus the increase in rate in a more acidic environment seems to be a characteristic property of the basic epoxides.*

Among the compounds discussed above the parent piperazine bis-epoxides (II) and the bipiperidyl analogue (V), water soluble solids melting at 43° and 97°, respectively, appear to be suitable candidates for chemotherapeutic use, with V possessing perhaps the advantage of greater activity and more suitable physical properties. To our knowledge, medicinals containing the 4,4′-bipiperidyl system have thus far not been reported to possess special merits. Thus, N-diethylcarbamoyl-N′-methyl-4,4′-bipiperidyl was found to be less effective as a filaricide *in vivo*[5] than the parent piperazine analogue, diethylcarbamizine. In our laboratories, the anthelmintic activity* of the base, 4,4′-bipiperidyl, was found to be negligible when compared to the potent oxyuricidal action of piperazine hexahydrate. Finally, within the class of alkylating agents itself, the anti-leukaemic activity of N,N′-bis(β-chloroethyl)-4,4′-bipiperidyl was determined to be only about one-third† of that of the piperazine analogue.[6] Thus, it appears that the enhanced anti-leukaemic activity of the bipiperidyl derivative (V) is characteristic for the present group of bis-epoxides.

Clinical studies‡ with the piperazine compound (II) have been

* These anthelmintic tests were performed by Mr. Max McCowen and associates of the Lilly Parasitological Research Group against *Aspicularis tetraptera* and *Syphacia obvalata* using the procedure by M. McCowen, M. Callender and M. Brandt, *Amer. J. Trop. Med. Hyg.*, **6**, 894 (1957)

† This observation indicates that merely increasing the distance between the alkylating functions does not necessarily lead to increased biological activity. In fact, it is recognized that the present work with basic bis-epoxides does not really allow a distinction between (a) the effect on biological activity of chain-length variations and (b) the effect of increased basicity for which these variations constitute the physical basis. In the earlier work of Ross[1] and Hendry[2] with non-basic bis-epoxides there are several indications that an increase in chain length is accompanied by a decrease rather than by an increase in biological activity. A comparison of the activities of X and XII may likewise be interpreted as emphasizing the role of base strength.

‡ These studies have been conducted by Drs. I. H. Krakow, D. G. Miller, D. A. Karnofsky, J. H. Burchenal, H. D. Diamond and L. F. Craver, Division of Clinical Chemotherapy, Sloan-Kettering Institute, the Department of Medicine of Memorial and James Ewing Hospitals, and Cornell Medical College, New York. A preliminary report of these studies was presented at the 7th International Congress of Cancer, July 9, 1958, London and will be published in the *ACTA* of the International Union against Cancer.

in progress for some time and similar studies with the bipiperidyl derivative (V) are now under way. A further report describing the biological evaluation of these compounds will be published shortly.*

Experimental

Di-secondary amines. Anhydrous piperazine of high purity was obtained in the form of white flakes from Dow Chemical Company. *Trans*-2,5-Dimethylpiperazine was procured from Carbide and Carbon Chemical Corporation. The N,N′-dimethyl derivatives of ethylene-, trimethylene-, tetramethylene-, and hexamethylenediamine were prepared by the procedure of Boon.[7] N,N-Dimethyl-tetramethylenediamine was also, more conveniently, obtained by catalytic reduction of 1,4-bis(methylamino)-2-butyne.[8]

4,4′-Bipiperidyl was prepared from pyridine by the method of Wibaut and Arens[9] using platinum oxide and acetic anhydride which had been purified by distillation in the presence of chromium trioxide; hydrolysis of the resulting N,N′-diacetyl bipiperidyl with concentrated hydrochloric acid provided a final product of higher purity than was obtained with alkaline methanolysis.[9] For larger quantities the chemical reduction method using zinc and acetic anhydride (non-purified)[10] was found to be more convenient. Final purification yielding anhydrous, carbonate-free bipiperidyl was effected by sublimation *in vacuo*[5] or recrystallization from benzene–petroleum ether mixtures.

4,4′-Bipiperidylmethane hydrochloride (m.p. 250°) was prepared by the catalytic reduction of 4,4′-bipyridylmethane[11] with platinum oxide in aqueous hydrochloric acid; the free base (m.p. 78°) was purified by sublimation *in vacuo*.

Anal. Calcd. for $C_{11}H_{22}N_2$: N, 15·37; mol. wt., 182. Found: N, 15·40; mol. wt. (electrometric titration), 180.

* Acknowledgment is gratefully made to Drs. Nelson J. Leonard and Henry A. Lardy, for helpful suggestions; Mr. William L. Brown and associates for the micro-analyses; Mr. Paul W. Landis, for electrometric titrations and other physical chemical aid; Miss Ann Van Camp, for numerous x-ray diffraction patterns; and Messrs James B. Leary and Earl Brown for epoxide titrations.

The execution of these studies was greatly aided by the interest and encouragement of Dr. R. G. Jones. For this help the authors wish to express their gratitude.

1,2-bi-(4-Piperidyl)ethane[12] was prepared by catalytic reduction of commercial 4,4′-dipyridylethylene (Aldrich Chemical Company) and purified by sublimation *in vacuo* (m.p. 112–113°).

The pK'_a values of the di-secondary amines used are listed in Table I (figures in parentheses).

N,N′-bis(2,3-Epoxy-n-*propyl)piperazine* (*II*). The preparation of this bis-epoxide is representative of the general procedure used and will be described in detail below; minor changes in the procedure used for the other bis-epoxides are mentioned in the sequel.

In a 2 l. three-necked round-bottomed flask provided with efficient stirrer and submerged thermometer was placed 86 g (1 mole) of anhydrous piperazine and 140 ml of absolute ethanol. The suspension was stirred and solution was achieved by heating with a warm water bath to about 30°. Epichlorohydrin (185 g, 2 moles) was added in one batch and the temperature was maintained between *30 and 35*° by means of occasional cooling with an iced-water bath. When heat evolution had subsided (about 45 min), the mixture was stirred vigorously for 1½ h. Occasionally, stirring was interrupted to break up the solid cake of bis-chlorohydrin formed. (This intermediate and the bis-chlorohydrin formed in the preparation of V are described in a later section). Anhydrous ether (200 ml) was added and the suspension was stirred *vigorously* during the drop-wise addition of 170 ml of an aqueous solution containing 85 g (2·1 moles) of sodium hydroxide. During the addition the temperature was kept below 25°, stirring being continued for ½ h afterwards. The solids were allowed to settle, and the supernatant alcohol–ether layer was decanted. The remaining solids were washed three times thoroughly with 200 ml of anhydrous ether and the combined ether extracts were then dried with the aid of solid potassium hydroxide pellets and sodium sulphate for ½ h. During this time the flask was swirled occasionally and kept in an ice bath. At this stage and throughout the remainder of the preparation the product was protected as much as possible from moisture in the air; filtrations described below were mostly carried out in a refrigerated room. The supernatant was decanted, the solids were washed with 100 ml of anhydrous ether and the combined ether solutions dried for ½ h in the same manner. This process

was repeated once more and the final solution filtered with the aid of charcoal. At this stage the solution was clear and colourless.

The solution was transferred to a round-bottomed flask and the main portion of the ether was removed by distillation *in vacuo* at room temperature until incipient crystallization. Crystallization was allowed to proceed to completion by cooling the contents to about $-50°$. After 1 h the crude product was filtered with suction on a Büchner filter, the residue in the flask was removed with 100 ml of cold ($-40°$) ether and added to the filter. A rubber filter dam was used to complete the filtration. The solids were transferred rapidly to a vacuum desiccator provided with calcium chloride and dried for 12 h at 0°. The preparation up to this point was carried out in one day as interruption at any stage, particularly before the crystallization, leads to a lowering of both the quality and the quantity of the final product.

Alternately, the crude product was obtained by removing all solvents and rapid distillation of the residue, collecting the fraction distilling between 110 and 120° at a pressure of about 0·03 mm. The distillate was obtained as a white, low-melting solid only if care was taken to interrupt the distillation when the contents of the flask turned from straw-yellow to light brown. Continuation beyond this point invariably led to violent, sometimes explosive, decomposition and to total loss of material.

The crude product, obtained by either crystallization or distillation, was dissolved in 1 l. of anhydrous ether; any undissolved material was removed by filtration. The filtrate was concentrated to about 200–300 ml and cooled to $-50°$. The crystalline material thus obtained was re-crystallized once more in the same manner, thoroughly dried under reduced pressure and stored at 0°. The weight of bis-epoxide (II) melting at 42–43° was 80 g (40 per cent); a sharp x-ray diffraction pattern gave evidence of the crystallinity of the material. After prolonged storage at 0° the epoxide content (96–97 per cent) decreased by a few per cent; small amounts of polymeric impurities could be removed by filtration and re-crystallization from ether, thereby restoring the initial purity of the product. The pure, dry product is only moderately hygroscopic.

Isolation of 'meso-isomer'(IIi). Occasionally, in the preparation of II complete solution was not achieved at the last re-crystallization. The insoluble material (10–20 g) was collected by filtration, dissolved in 300–400 ml of anhydrous ether by slight warming and allowed to crystallize at $-10°$. A second re-crystallization from ether gave a white, crystalline isomer melting at 62–63°. The x-ray diffraction pattern of this isomer differed from that of the main product (II); the molecular weight determined cryoscopically in acetone was 205 (calculated 198).

The acute intravenous toxicity in mice of the main product (II) and of the '*meso*-isomer'(IIi) expressed as LD50 were determined to be 85 ± 8 mg/kg, and 77 ± 6 mg/kg, respectively.

Other bis-epoxides. Preparation of the other bis-epoxides proceeded mainly in the manner described for II with yields of the final product ranging from 20 to 50 per cent. In small scale preparations it was possible to control the initial condensation reaction in the temperature range of 35–40°. The liquid bis-epoxides (IX through XII) were distilled two or three times with boiling points ranging from 110 to 130° at pressures of 0·05 to 0·2 mm. Epoxide values are listed in Table I, while physical constants and analytical data are given in Table II.

The preparation of the 4,4′-bipiperidyl derivative (V) was hampered by low solubility of the intermediate bis-chlorohydrin, but an acceptable yield (45 per cent) was obtained when methanol was used as the solvent. Surprisingly, the acute intravenous toxicity of V in mice was found to be of the same order (LD_{50} 74 ± 7 mg/kg) as that of II.

N,N-bis(2,3-Epoxypropyl)methylamine (Ia, b.p. 97–99°/7 mm) and tris(2,3-epoxypropyl)amine (Ib, b.p. 158–160°/7 mm) were prepared in 30–40 per cent yield using ice-cooled methanolic solutions (75 ml) containing 0·5 mole of methylamine and ammonia gas, respectively. After addition of epichlorohydrin (1 and 1·5 moles, respectively), the mixture was stirred and the temperature allowed to rise to and then maintained at about 40°. Loss of the volatile bases was prevented by the use of a Dry-ice condenser. After the initial heat evolution had subsided (2 to 3 h), the mixture was stirred at room temperature for 18 h. Treatment with alkali as described above and distillation of the residue from the ether solution gave the clear, colourless bis- and

Table II

Compound	n_D^{25} or m.p., °C	Formula	Analyses, % carbon calcd.	carbon found	hydrogen calcd.	hydrogen found	nitrogen calcd.	nitrogen found	Mol. wt.* calcd.	Mol. wt.* found
Ia	1·4529	$C_7H_{13}NO_2$					9·79	9·13	143	155
Ib	1·4727	$C_9H_{15}NO_3$					7·55	7·90	185	176
II	42–43	$C_{10}H_{18}N_2O_2$	60·58	60·67	9·15	9·43	14·13	13·88	198	201
IIi	62–63	$C_{10}H_{18}N_2O_2$					14·13	13·91	198	202
III	82–83	$C_{12}H_{22}N_2O_2$	63·68	64·26	9·80	9·72	12·38	12·18	226	225
IV	83–85	$C_{12}H_{22}N_2O_2$	63·68	63·96	9·80	9·68	12·38	12·34	226	244
V	96–98	$C_{16}H_{28}N_2O_2$	68·53	68·28	10·07	9·90	9·99	9·79	280	276
VI	56–58	$C_{17}H_{30}N_2O_2$	69·34	68·69	10·27	10·55	9·52	10·10	294	283
VII	86–88	$C_{18}H_{32}N_2O_2$	70·09	70·26	10·46	10·41	9·08	8·72	308	304
VIII	†	$C_{10}H_{20}N_2O_2$					14·00	14·18	200	—
IX	1·4660	$C_{11}H_{22}N_2O_2$					13·07	12·50	214	228
X	1·4662	$C_{12}H_{24}N_2O_2$	63·12	63·15	10·60	10·79	12·27	12·15	228	244
XI	1·4670	$C_{14}H_{28}N_2O_2$	65·58	65·22	11·01	11·34	10·93	10·72	256	298
XII	1·4858	$C_{12}H_{20}N_2O_2$					12·50	12·32	224	220

* Molecular weight determinations by electrometric titration in 66 per cent dimethylformamide.
† See text.

tris-epoxide, respectively. These epoxides (Ia and Ib) are considerably more stable than the other liquid basic bis-epoxides. Even the crude product can be distilled without risk of decomposition, but yellow, polymeric material deposits after several weeks of storage in the refrigerator.

In attempts to prepare the ethylenediamine derivative (VIII), the initially clear ethereal solutions rapidly became turbid. When trying to distill the residue, a vigorous reaction ensued evolving enough heat to distill the product without external heating (vapour pressure about 110° at 0·1–0·5 mm). The clear yellow distillate, n_D^{25} 1·4750, showed a prominent infrared absorption band at 6·05 μ. After 24 h, the material had become dark and very viscous (n_D^{25} 1·4970) and could not be distilled.

bis-Chlorohydrin intermediates. These above-mentioned intermediates were isolated for the purpose of characterization in the course of the preparation of the piperazine (II) and bipiperidyl bis-epoxide (V). Re-crystallization from ethanol resulted in low recovery, presumably due to instability of basic chlorohydrins of this type. N,N′-Bis(3-chloro-2-hydroxy-*n*-propyl)piperazine melted at 125–127°.

Anal. Calcd. for $C_{10}H_{20}Cl_2N_2O_2$: N, 10·33. Found: N, 10·35.

The *dihydrochloride*, prepared in ethanol and re-crystallized from the same solvent with good recovery, melted at 215–200°.

Anal. Calcd. for $C_{10}H_{20}Cl_2N_2O_2 \cdot 2HCl$: C, 34·90; H, 6·44; N, 8·14. Found: C, 34·99; H, 6·69; N, 7·99.

N,N′-bis(3-Chloro-2-hydroxy-n-*propyl-4,4′-bipiperidyl* melted at 220° with decomposition.

Anal. Calcd. for $C_{16}H_{30}Cl_2N_2O_2$: C, 54·38; H, 8·56; N, 7·93; mol. wt., 353. Found: C, 54·01; H, 8·25; N, 7·76; mol. wt. (electrometric titration), 363.

The *dihydrochloride* melted at 245–250°.

Anal. Calcd. for $C_{16}H_{30}Cl_2N_2O_2 \cdot 2HCl$: C, 45·03; H, 7·63; N, 6·57; mol. wt., 426. Found: C, 45·02; H, 7·78; N, 6·18; mol. wt. (electrometric titration), 408.

N,N′-bis(2,3-Dihydroxy-n-*propyl)piperazine* (see footnote * page 225). To a solution of 8·6 g (0·1 mole) of piperazine in 20 ml of ethanol, 15 g (0·2 mole) of glycidol was added in one batch. The solution was stirred and the temperature maintained between 25 and 30°. After the heat evolution had subsided, stirring was

continued for ½ h and the solid which had crystallized out was filtered and washed three times with 10 ml of cold ethanol. The tetrol could not be recrystallized from hot ethanol or methanol because of low solubility and was therefore purified by digestion in hot 95 per cent ethanol for 1 h. The hygroscopic product melted at 175–180°.

Anal. Calcd. for $C_{10}H_{22}N_2O_4$: N, 11·90; mol. wt., 234. Calcd. for monohydrate: N, 11·10; mol. wt., 252. Found: N, 10·95; mol. wt. (electrometric titration), 240.

The *dihydrochloride* crystallized from ethanol and melted at 225°.

Anal. Calcd. for $C_{10}H_{22}N_2O_4 \cdot 2HCl$: N, 9·11. Found: N, 9·38.

Periodate oxidation of this tetrol was carried out using the general procedure described.[13] The dihydrochloride (307 mg, 1 mm) was dissolved in about 10 ml of water and 40 ml of a 0·1 molar sodium metaperiodate solution (4 mmoles) was added. The solution was made up to 100 ml and 10 ml aliquots were titrated periodically with 0·02 *N* sodium arsenite solution.

Time (min.)		3–4	15	30	60
Arsenite, ml.	sample	18·30	9·0	3·5	2·8
	blank	39·0	39·5	38·1	38·5
Moles IO_4^-/mole of tetrol		2·1	3·1	3·74	3·85

Base hydrolysis of II. A solution of 1·58 g (8 mmoles) of the piperazine bis-epoxide (II) in 100 ml of 2 *N* NaOH was allowed to stand at room temperature for 48 h. Two hundred ml of 0·1 molar sodium meta-periodate solution (20 mmoles) and 225 ml of 1 *N* hydrochloric acid was added and the solution made up to 1 l. The solution which had a pH of 2·8 was allowed to stand for 1 h at room temperature in the dark. A 100 ml aliquot was added to 500 ml of a saturated aqueous solution of dimedone. After standing at room temperature for 24 h (pH 4·0), the formaldehyde derivative was filtered off with suction, washed twice with 100 ml of cold water, and dried *in vacuo* at 60°. The x-ray diffraction pattern of this material, m.p. 189°, was identical

with that of an authentic sample of formaldehyde-dimedone derivative. The weight of dried material was 402 mg (87 per cent). A separate experiment had previously established the feasibility of making the dimedon derivative in the presence of excess iodate ion.

Reaction of bis-epoxides II, V, VII, and XIII with KH_2PO_4, K_2HPO_4, and KH_2PO_4–K_2HPO_4 mixtures. Samples of the bis-epoxides (0·75 moles) were dissolved in 10 ml of an aqueous phosphate solution containing 1·5 mmoles of one of the above salts (261 mg and 204 mg, respectively) or a total of 1·5 mmoles of a mixture of the above salts in a proportion providing the desired pH (about 7·2) after addition of the particular bis-epoxide under investigation. The flasks were stored in a constant temperature bath at 37° C and aliquots were removed periodically for the determination of phosphate ion. Each aliquot was diluted to 25 ml with water and a 1 ml aliquot of the resulting solution was allowed to react with phosphomolybdic reagent. Inorganic phosphate was determined by the method of Bernhart and Wreath.[14] A blank was run alongside each determination using 1 ml of the particular stock phosphate solution used.

The pH of the reaction mixture was determined at the time of mixing and after 24 h. In a parallel experiment the epoxide content of some of the final solutions, collected after 24 h, was determined for the reaction mixtures of the bis-epoxides (II, V, and XIII) with the pure salts, KH_2PO_4 and K_2HPO_4, respectively. The epoxide contents found are listed, together with the phosphorolysis data in Table III. It was found, however, in a control experiment that phosphate ion interfered with the thiosulphate assay used, resulting in values lowered to the extent of 2–5 per cent. The epoxide values listed, therefore, merely represent minimum values. Consequently, the extent to which hydrolysis competes in these mixtures with phosphorolysis[1] could be estimated (last column) but not determined accurately.

N,N′-bis(β-Chloroethyl)-4,4′-bipiperidyl. This 'split' nitrogen-mustard was prepared by the procedure[6] used for the piperazine analogue. N,N′-Bis(β-hydroxyethyl)-4,4′-bipiperidyl dihydrochloride melted at 200–225°.

Anal. Calcd. for $C_{14}H_{28}N_2O_2 \cdot 2HCl$: N, 8·51. Found: N, 8·22.

Table III. Reaction of bis-epoxides (II, V, VII, and XIII) with phosphate ion

Compound	Phosphate	Time, h → 0 pH	1	3	6	24	pH	Epoxide, %†	Hydrolysis, %‡
			% Phosphorolysis						
Blank	KH_2PO_4	4·2	←——0±2——→				4·2		
	K_2HPO_4	9·2					9·2		
II	KH_2PO_4	6·3	10	13	15	36	6·4	41	23
	Mixture*	7·1	—	—	12	27	7·5	—	—
	K_2HPO_4	9·3	2	7	10	18	10·9	31	51
V	KH_2PO_4*	7·2	12	24	40	63	7·8	10	27
	K_2HPO_4	9·9	4	7	15	35	11·0	23	42
VII	KH_2PO_4	7·6	—	13	37	63	8·5	—	—
XIII	KH_2PO_4	4·2	—	0	—	2?	4·25	71	28
	Mixture*	7·2	—	3	—	13	7·5	—	—
	K_2HPO_4	9·2	3	7	10	28	10·9	16	56

* Represented in Fig. 1.
† Represents a minimum value requiring a correction of plus 2–5 per cent (see Experimental text).
‡ Similarly, this figure is a maximum value requiring a correction of minus 2–5 per cent.

The dihydrochloride of the N,N′-bis(β-chloroethyl)-derivative turned brown at 250° but did not melt up to 285°.

Anal. Calcd. for $C_{14}H_{26}N_2Cl_2.2HCl$: Cl, 38·08. Found: Cl, 37·69.

Summary. The preparation, properties, and anti-leukaemic activity of a group of N,N′-bis(2,3-epoxypropyl) substituted bis-secondary amines are described. The relation between base strength of the bis-epoxides and their anti-leukaemic effectiveness was studied. The piperazine (II) and 4,4′-bipiperidyl derivative (V) are of interest as potential chemotherapeutic agents.

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