activity by a procedure similar to that of Aceto and Harris[21] as modified by Nematollahi, *et al.*[22] A dose of 1.1 mmoles/kg was administered orally to groups of ten mice (male, strain C_3H 20 ± 1 g). Since IX and XIII are insoluble in water, a 3% suspension of acacia in distilled water was used as the vehicle in all cases except where otherwise noted. After 2 hr, five mice in each group were challenged with a 2.5-mg/kg ip dose of reserpine; the other five being given 5 mg/kg ip of reserpine. The animals were left undisturbed for 3 hr then evaluated as to degree of blepharoptosis by two individuals. Ten control mice received only vehicle and reserpine (2.5 and 5 mg/kg for each half) as a control while isocarboxazide, administered in the same manner as the test compounds at a dose of 0.275 mmole/kg, served as a standard. The following ptotic scoring system of Aceto and Harris[21] was used: 4 = complete, 3 = three-fourths, 2 = one-half, 1 = one-fourth closure of the eyelids and 0 = open eyelids. Since the administration of XI resulted in convulsive death of all animals at the 1.1-mmoles/kg level, the test was repeated using 0.275, 0.55, and 0.825-mole/kg doses (aqueous solutions) in a group of five mice with a challenging dose of 5 mg/kg ip of reserpine. The results of the testing are given in Table III.

(21) M. D. Aceto and L. P. Harris, *J. Toxicol. Appl. Pharmacol.*, **7**, 329 (1952).

(22) J. Nematollahi, W. Guess, and J. Autian, *J. Med. Chem.*, **9**, 660 (1966).

Anticancer Screening.—Derivatives containing aziridinyl moieties were tested against Walker 256 subcutaneous (WA); the remaining compounds against Walker 256 intramuscular (WM) and/or lymphoid leukemia L1210 (LE).[23] Screening results are presented in Table IV.

Antimalarial Screening.—Five mice were infected with a lethal dose of *Plasmodium berghei* 3 days prior to administration of I, II, VII–X, and XII in doses of 40, 160, and 640 mg/kg sc in oil.[24] The mean survival time of infected control mice was 6.1 days. The changes in survival time are given as the mean survival time of the treated group minus the mean survival time of the control group. The results of the rodent antimalarial tests are given in Table IV.

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(23) Test results furnished by the Cancer Chemotherapy National Service Center, Bethesda, Md.

(24) Test results furnished by Walter Reed Army Medical Center, Washington, D. C.

Alkylating Agents Related to 2,2′-Biaziridine. II.[1] N,N′-Dicarbethoxy-2,2′-biaziridine

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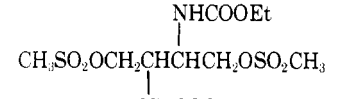
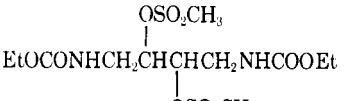
DL- and (2*R*:2′*R*)-N,N′-dicarbethoxy-2,2′-biaziridine (**3**) and the bismethanesulfonates of the symmetrical position isomers of DL- and (2*S*:3*S*)-N,N′-dicarbethoxydiaminobutanediols (**1** and **2**) were synthesized. Unsuccessful attempts to prepare the corresponding 1,4-dihalogeno-2,3-diaminobutanes are mentioned. As far as they are available the anticancer screening data for the compounds are summarized.

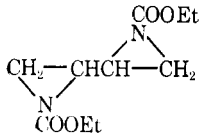
For reasons already discussed[1] the present communication concerns the synthesis of DL- and (2*S*:3*S*)-N,N′-dicarbethoxy-2,3-diamino-1,4-butanediol 1,4-bismethanesulfonate [DL-**1**, (*S*:*S*)-**1**], (2*S*:3*S*)-N,N′-dicarbethoxy-1,4-diamino-2,3-butanediol 2,3-bismethanesulfonate [(*S*:*S*)-**2**], and DL- and (2*R*:2′*R*)-N,N′-dicarbethoxy-2,2′-biaziridine [DL-**3**, (*R*:*R*)-**3**].



1



2



3

(1) Paper I: P. W. Feit and O. Tvaermose Nielsen, *J. Med. Chem.*, **10**, 697 (1967).

The biaziridine derivatives **3** might act *in vivo* as alkylating agents *per se*, or alternatively after being metabolized to the corresponding unsubstituted 2,2′-biaziridine, inasmuch as James[2] has proved aziridine formation from N-carboalkoxyaziridines *in vitro* after incubation with rat plasma.

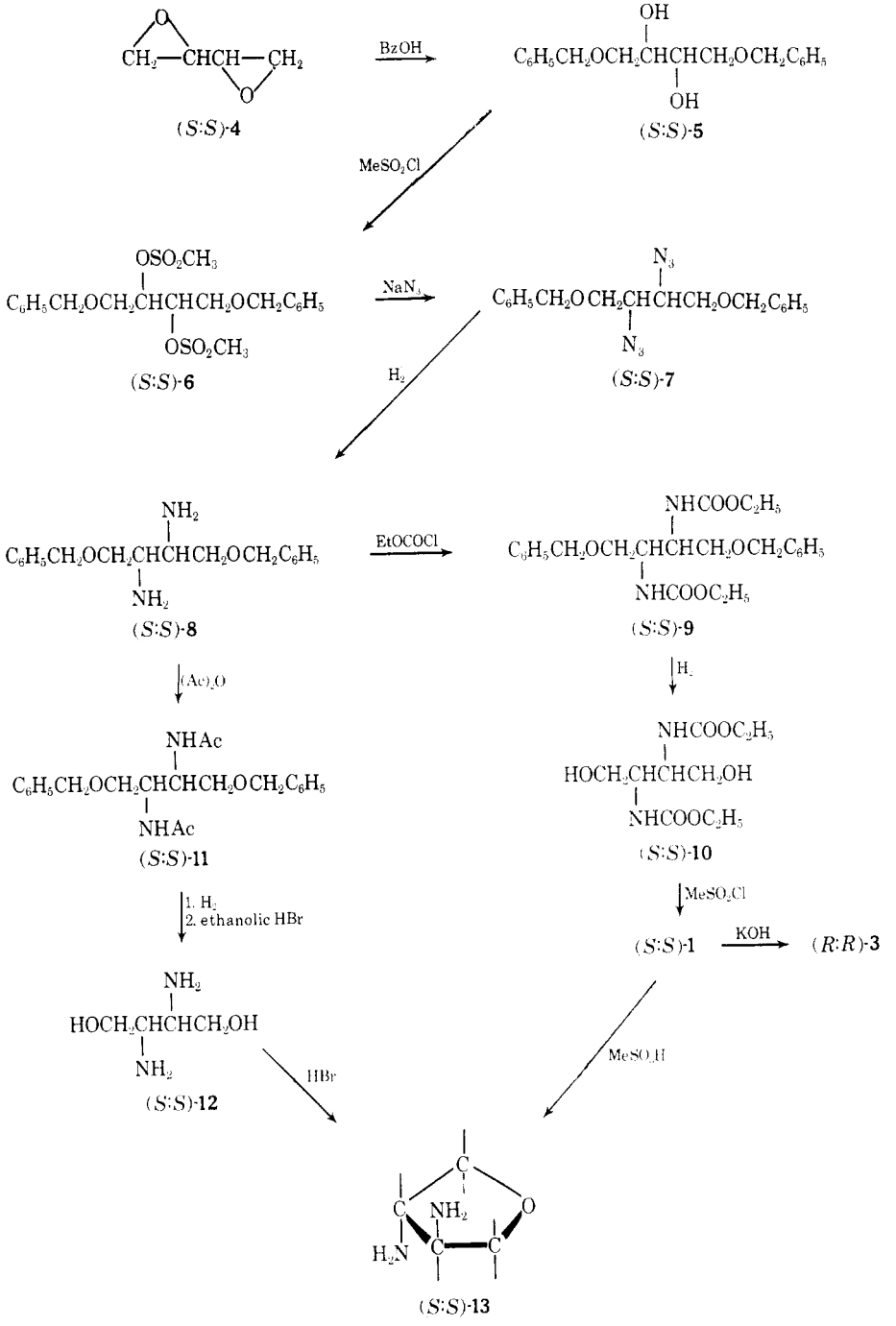
For the N,N′-dicarbethoxydiaminobutanediol bismethanesulfonates **1** and **2** a ring closure to the biaziridine **3** seems unlikely under physiological pH as this reaction proceeds *in vitro* under strong alkaline conditions only. However, there might be a possibility of metabolizing to the unsubstituted amino compounds which are considered *in vivo* precursors of the corresponding 2,2′-biaziridine.

Chemistry.—The synthetic route for the preparation of DL-**1** and (*S*:*S*)-**1** and the corresponding biaziridines DL-**3** and (*R*:*R*)-**3** is summarized for the optically active compounds in Scheme I.[3] Ring closure to the biaziridine (*R*:*R*)-**3** could be performed by treatment of (*S*:*S*)-**1** with KOH in methylene chloride. Examination of the infrared spectrum established the structure since a strong carbonyl absorption is present while

(2) R. M. James, *Biochem. Pharmacol.*, **14**, 915 (1965).

(3) The reaction from **6** to **7** proceeds under Walden inversion at C-2 and -3. The prefix (*S*:*S*) is not changed in this special case due to the rules of the Cahn–Ingold–Prelog system: R. S. Cahn, C. K. Ingold, and V. Prelog, *Angew. Chem.*, **78**, 413 (1966), and preceding papers.

SCHEME I



the NH bond and amide-II bond are lacking. The conversion of β-iodo carbamates to aziridines, and the isolation of intermediately formed N-carbalkoxyaziridine after treatment of methyl (3α-iodo-2β-cholestane)-carbamate with methanolic KOH as described and discussed by Hassner and Heathcock[4] have encouraged us to attempt this ring closure reaction.

meso-**8**, DL-**8**, and (*S*:*S*)-**8** served as starting material for the preparation of the corresponding 2,3-diamino-1,4-butanediol's (**12**). Reductive debenzylation of **8** to **12** failed in our hands, but could be performed after acetylation to the N,N'-diacetyl compounds **11**, and subsequent deacylation. The structure seems to be provided by the synthetic route, but could be further established as $LiAlH_4$ reduction of diethyl *meso*-2,3-dibenzylaminosuccinate resulted in *meso*-2,3-dibenzylamino-1,4-butanediol, which after reductive debenzylation gave *meso*-**12**. Attempts to prepare the 1,4-dichloro-2,3-diaminobutane dihydrochlorides from the corresponding **12**·2HCl by means of thionyl chloride were unsuccessful, although this reaction could be performed with the *meso*-dibenzylamino compound to give *meso*-1,4-dichloro-2,3-dibenzylaminobutane dihydrochloride. Unfortunately, we were unable to debenzylate this compound. Reaction with $SOCl_2$ of DL-2,3-bis-(dimethylamino)-1,4-butanediol dihydrochloride prepared by reductive alkylation of DL-**12**·2HCl resulted in the cyclic sulfite.

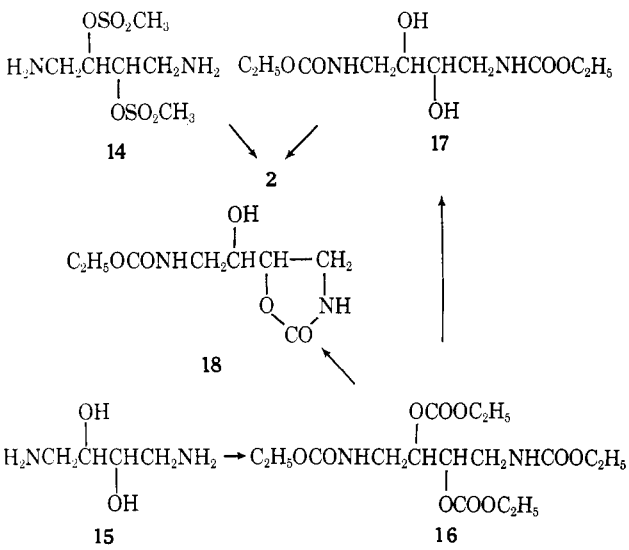
Treatment of (*S*:*S*)-**1** in 90% methanesulfonic acid under various conditions by analogy with the described[1]

(4) A. Hassner and C. Heathcock, *Tetrahedron*, **20**, 1037 (1964); *J. Org. Chem.*, **29**, 3640 (1964).

hydrolysis of 1,4-bisphthalimido-2,3-butanediol 2,3-bismethanesulfonate did not give the desired 2,3-diamino-1,4-butanediol 1,4-bismethanesulfonate, but yielded the tetrahydrofuran (*S*:*S*)-**13**. The physical properties including the optical rotation of the dihydrobromide of (*S*:*S*)-**13** were identical with those of the compound prepared by an independent route.[5]

DL- and (2*S*:3*S*)-N,N′-dicarbethoxy-1,4-diamino-2,3-butanediol 2,3-bismethanesulfonate [DL-**2**, (*S*:*S*)-**2**] were available by carbethoxylation of the corresponding diamine **14** or from (2*S*:3*S*)-1,4-diamino-2,3-butanediol [(*S*:*S*)-**15**] as summarized in Scheme II. The saponification of (*S*:*S*)-**16** was accompanied by a cyclization reaction as the oxazolidone (*S*:*S*)-**18**[6] was isolable from the reaction mixture.

SCHEME II



Anticancer Screening.—Compounds DL-**1**, (*S*:*S*)-**1**, (*S*:*S*)-**2**, DL-**3**, and *meso*-1,4-dichloro-2,3-dibenzylaminobutane dihydrochloride were submitted for screening to the Cancer Chemotherapy National Service Center, National Institutes of Health. DL-**1**, (*S*:*S*)-**1**, and (*S*:*S*)-**2** when tested in the KB cell culture system and the Carcinoma 1025 system (mice) lacked antitumor activity even when administered intraperitoneally once daily with 500 mg/kg for 5 days in the latter system. The dichlorodibenzylaminobutane compound was inactive in the Walker carcinosarcoma 256 (subcutaneous) system in doses of 100 mg/kg once daily for 5 days. For DL-**3** a summary of the test data are presented in Table I, indicating that this compound is relatively toxic and has no significantly selective antitumor activity when tested in this manner.

TABLE I

SCREENING DATA FOR DL-N,N′-DICARBETHOXY-2,2′-BIAZIRIDINE (DL-**3**)

Test system	Daily dose, mg/kg[a]	Survivors	Mean tumor wt test/control, %[b]
Toxic test[c]	50	0/3	
	10	0/3	
	3	3/3	
Walker carcinosarcoma 256 (subcutaneous)[d]	1	6/6	52
Walker carcinosarcoma 256/cytoxan (subcutanous)[d]	3.8	6/6	71
	1.9	6/6	87
	0.95	6/6	97
	0.47	6/6	96

[a] Administered intraperitoneally once daily, days 1–5. [b] Sacrificed and evaluated 10 days postinoculation. [c] Fischer 344 rats, evaluated 10 days after first injection. [d] Random bred albino rats.

Experimental Section[7]

1,4-Dibenzyloxy-2,3-butanediols (5). Method A (Table II).—To a solution of sodium benzyloxide (13 g) in benzyl alcohol (300 g), **4** (43.1 g) was added dropwise over a period over about 30 min while stirring at 55–85°. The mixture was left for about 20 hr, then diluted with diethyl ether (500 ml) and washed with 1 *N* H_2SO_4 and with water. Diethyl ether and excess benzyl alcohol were removed under reduced pressure. The remaining crude **5** (130–140 g), which solidified on cooling, was sufficiently pure for further reaction.

1,4-Dibenzyloxy-2,3-butanediol 2,3-Bismethanesulfonates (6). Method B (Table II).—To a solution of **5** (134 g) in pyridine (400 ml), methanesulfonyl chloride (125 ml) was added dropwise over a period over 4.5 hr while stirring at 6–13°. The mixture was kept in a refrigerator for 20 hr and then poured into ice–2 *N* HCl (2.5 l.). The resulting precipitate[8] was washed with water and with ethanol and triturated with ethanol (500 ml) to give 180–200 g of crude **6**.

2,3-Diamino-1,4-dibenzyloxybutane Dihydrochlorides (8·2HCl). Method C (Table II).—A suspension of **6** (114.5 g) and NaN_3 (100 g) in formamide (400 ml) was stirred at 110–112° for about 17 hr. After dilution with water (1 l.) the mixture was extracted with diethyl ether (500 ml); the extract was washed with saturated NaCl solution (100 ml), dried ($MgSO_4$), and evaporated under reduced pressure. The resulting crude **7** (about 70 g) in ethanol (300 ml) was hydrogenated in the presence of 10% Pd–$CaCO_3$ (8 g) by blowing H_2 through the vigorously stirred reaction mixture. During the hydrogenation the temperature rose to about 60°. After about 4 hr the mixture had again reached room temperature, and an evaporated sample lacked an infrared azide absorption band at 2120 cm^{-1} ($CHCl_3$), indicating that the hydrogenation was complete. The catalyst was removed by filtration and the resulting clear solution was acidified with dry HCl. After concentration under reduced pressure to about 100 ml the precipitate was filtered off and washed with ethanol and diethyl ether to give 24–30 g of crude **8**·2HCl.

N,N′-Dicarbethoxy-2,3-diamino-1,4-butanediols (10). Method D (Table II).—To a solution of **8**·2HCl (45 g) and NaOH (25 g) in water (350 ml), ethyl chloroformate (35 ml) was added dropwise over a period of about 30 min while stirring at 0–5°. After additional stirring for about 1 hr concentrated HCl (20 ml) was added and the mixture was extracted with diethyl ether. The extract was dried ($MgSO_4$) and evaporated under reduced pressure. The resulting **9**[9] (50–60 g) in ethanol (500 ml) acidified with concentrated HCl (10 ml) was hydrogenated in the presence of 10% Pd–C (8 g) at 1.1 atm. The hydrogen uptake was complete in about 2 hr. The catalyst was removed by filtration and the resulting solution was evaporated under reduced pressure. Trituration with diethyl ether gave 20–25 g of crude **10**.

N,N′-Dicarbethoxy-2,3-diamino-1,4-butanediol 1,4-Bismethanesulfonates (1). Method E (Table II).—To a solution of **10** (20 g) in pyridine (100 ml), methanesulfonyl chloride (15 ml)

(5) P. W. Feit and O. Tvaermose Nielsen, publication in preparation.

(6) (*S*:*S*)-**18** shows infrared carbonyl absorption bands at 1711 and 1761 cm^{-1} ($CHCl_3$), making a difference between the absorption frequencies of the oxazolidone ring carbonyl and its acyclic analog, both present in this molecule of 50 cm^{-1}. H. K. Hall, Jr., and R. Zbinden, *J. Am. Chem. Soc.*, **80**, 6428 (1958), measured a difference of 52 cm^{-1} between the corresponding frequencies of a five-membered ring and its acyclic urethan analog.

(7) Analyses are by G. Cornali and W. Egger of these laboratories. Melting points were taken in open glass capillaries and rounded off to half degrees, using a Hershberg apparatus with thermometers subdivided into 0.1°.

(8) (*S*:*S*)-**6** separated as an oil, which was extracted with $CHCl_3$, dried ($MgSO_4$), evaporated under reduced pressure, and solidified with ethanol.

(9) Only (*S*:*S*)-**9** was obtained in crystalline form with mp 71–72° (diethyl ether–heptane), $[\alpha]^{20}D$ −14.5° (*c* 2, ethanol). *Anal.* Calcd for $C_{24}H_{32}N_2O_6$: C, 64.84; H, 7.26; N, 6.30. Found: C, 64.74; H, 7.18; N, 6.28.

TABLE II
$XCH_2CHYCHYCH_2X$

No. and config	X	Y	Mp, °C	Solvent of recrystn	$[\alpha]^{25}D$, deg	Method[a]
DL-**1**	OSO_2CH_3	$NHCOOC_2H_5$	123.5–124	Acetone–water		E
(*S*:*S*)-**1**	OSO_2CH_3	$NHCOOC_2H_5$	112–112.5	Ethanol	+23.2[b]	E
DL-**3**	$NCOOC_2H_5$		35.5–37	[c]		F
(*R*:*R*)-**3**	$NCOOC_2H_5$		54–55	[c]	+206[d]	F
meso-**5**	$OCH_2C_6H_5$	OH	56–57	[c]		A
DL-**5**	$OCH_2C_6H_5$	OH	61.5–62.5	[c]		A
(*S*:*S*)-**5**	$OCH_2C_6H_5$	OH	53.5–55	[c]	−3.4[d]	A
meso-**6**	$OCH_2C_6H_5$	OSO_2CH_3	101.5–103	Ethanol		B
DL-**6**	$OCH_2C_6H_5$	OSO_2CH_3	84.5–85	Ethanol		B
(*S*:*S*)-**6**	$OCH_2C_6H_5$	OSO_2CH_3	76.5–77.5	Ethanol	−11.7[d]	B
meso-**8**·2HCl	$OCH_2C_6H_5$	NH_2	231.5–232	Ethanol		C
DL-**8**·2HCl	$OCH_2C_6H_5$	NH_2	204.5–206	Ethanol		C
(*S*:*S*)-**8**·2HCl	$OCH_2C_6H_5$	NH_2	198.5–199.5	Ethanol	+14.5[f]	C
DL-**10**	OH	$NHCOOC_2H_5$	102.5–103.5	Ethanol–diethyl ether		D
(*S*:*S*)-**10**	OH	$NHCOOC_2H_5$	118–119	Ethanol–diethyl ether	+29.5[g]	D
meso-**11**	$OCH_2C_6H_5$	$NHCOCH_3$	178.5–179	Ethanol		G(a)
DL-**11**	$OCH_2C_6H_5$	$NHCOCH_3$	142.5–143	Ethyl acetate		G(b)
(*S*:*S*)-**11**	$OCH_2C_6H_5$	$NHCOCH_3$	137–137.5	Ethyl acetate	−33.1[g]	G(a), (b)
meso-**12**·2HBr[h]	OH	NH_2	214–215	90% ethanol		H(a), (b)
(D-**12**·2HBr[i]	OH	NH_2	183–184	Ethanol		H(a)
SL:*S*)-**12**·2HBr[j]	OH	NH_2	158.5–159	Ethanol	+6.2[f]	H(a)
meso-**12**·2HCl	OH	NH_2	241.5–242.5	Water–acetone		I
DL-**12**·2HCl	OH	NH_2	143.5–144.5	Methanol–diethyl ether		I
(*S*:*S*)-**12**·2HCl[k]	OH	NH_2	135–135.5	Methanol		I
DL-**13**·2HBr	X,X = O	NH_2	Dec from 250	48% HBr		K(a)
(*S*:*S*)-**13**·2HBr	X,X = O	NH_2	Dec from 270	48% HBr	−20.9[f,l]	K(a), (b)
meso-**19**·2HCl	OH	$N(CH_3)_2$	228.5–229	90% ethanol		J
DL-**19**·2HCl	OH	$N(CH_3)_2$	66.5–68	Ethanol		J
(*S*:*S*)-**19**·2HCl	OH	$N(CH_3)_2$	206–208	Ethanol	−21.0[f]	J

[a] The letters relate to the general procedures in the Experimental Section. [b] (*c* 2, acetonitrile). [c] Purified by redistillation *in vacuo* (0.5 mm, oil-bath temperature 170–190°). [d] (*c* 2, $CHCl_3$). [e] Purified by distillation *in vacuo* (0.2 mm, oil-bath temperature about 200°). [f] (*c* 2, water). [g] (*c* 2, EtOH). [h] The analytically pure bissalicylaldehyde Schiff base had mp 185–185.5° (MeOH). [i] The analytically pure bissalicylaldehyde Schiff base had mp 178–178.5° (MeOH). [j] The analytically pure bissalicylaldehyde Schiff base

was added dropwise over a period of about 30 min while stirring at 0–5°. After additional stirring for about 30 min the mixture was poured into ice–2.5 *N* HCl (400 ml). The resulting precipitate was washed with water, ethanol, and diethyl ether to give 20–24 g of crude **1**.

N,N′-Dicarbethoxy-2,2′-biaziridines (3). Method F (Table II).—A mixture of **1** (10.5 g) and finely grained KOH (11.5 g) in CH_2Cl_2 (100 ml) was stirred at room temperature for about 17 hr. The mixture was filtered (Dicalite) and the resulting clear solution was evaporated under reduced pressure. The residue (about 6 g) was distilled *in vacuo* (0.5 mm, oil-bath temperature 170–190°) to give about 3.5 g of **3**.

2,3-Diacetamido-1,4-dibenzyloxybutanes (11). Method G (Table II). (**a**)—To a suspension of **8**·2HCl (3.7 g) in 2 *N* NaOH (30 ml), acetic anhydride (5 ml) was rapidly added dropwise while stirring at 20–25°. After additional stirring for about 1 hr the precipitate was washed with water, ethanol, and diethyl ether to give about 3.3 g of crude **11**.

(**b**) A mixture of **8**·2HCl (18.7 g), pyridine (15 ml), and Ac_2O (40 ml) was heated on a steam bath for about 40 min. Excess Ac_2O was evaporated under reduced pressure and the residue triturated with water (200 ml) to give about 15 g of crude **11**.

2,3-Diamino-1,4-butanediol Dihydrobromides (12·2HBr). Method H (Table II). (**a**)—A solution of **11** (1.9 g) in ethanol (50 ml) acidified with 48% HBr (0.2 ml) was hydrogenated in the presence of 10% Pd–C (0.3 g) at 1.1 atm. The hydrogen uptake was complete in about 1 hr. The catalyst was removed by filtration and thoroughly washed with water. After addition of 48% HBr (5 ml) the combined filtrate was refluxed for about 2 hr and then evaporated under reduced pressure. Trituration with acetone gave 0.9 g of crude **12**·2HBr.

(**b**) A solution of *meso*-2,3-dibenzylamino-1,4-butanediol dihydrobromide (1.15 g) in water (40 ml), acidified with 48% HBr (0.1 ml), was hydrogenated in the presence of 10% Pd–C (0.4 g) at 1.1 atm. The hydrogen uptake was complete in about 2 hr. The catalyst was removed by filtration and the resulting solution was evaporated under reduced pressure. Trituration with acetone gave 0.7 g of crude *meso*-**12**·2HBr. The infrared spectrum (KBr) and the analysis were identical with those of the material prepared as in part a.

Method I (Table II).—The **dihydrochlorides** of **12** were prepared using method H(a) except that HBr was replaced by HCl.

The **bissalicylaldehyde Schiff bases** of **12** were prepared using the method of Carroll.[10]

Diethyl-*meso*-2,3-dibenzylaminosuccinate Dihydrochloride.—*meso*-2,3-Dibenzylaminosuccinic acid[11] (125 g) was esterified in hot ethanol saturated with dry HCl yielding crude dihydrochloride (90 g). An analytical sample was recrystallized from ethanol: mp 170.5–171.5° dec.

Anal. Calcd for $C_{22}H_{28}N_2O_4 \cdot 2HCl$: C, 57.77; H, 6.61; N, 6.13; Cl, 15.50. Found: C, 57.74; H, 6.81; N, 6.19; Cl, 15.30.

The **diester** was liberated from its dihydrochloride (86 g) by treatment with a slight excess of triethylamine (40 g) in water (600 ml). After washing with water and a small amount of ethanol, and drying *in vacuo* at 50° for about 20 hr crude diethyl *meso*-2,3-dibenzylaminosuccinate (65 g) with mp 73–76° was obtained. This material was sufficiently pure for further reaction.

***meso*-2,3-Dibenzylamino-1,4-butanediol Dihydrobromide.** A suspension of $LiAlH_4$ (23 g) in diethyl ether (500 ml) was refluxed for 45 min with vigorous stirring. A suspension of diethyl *meso*-2,3-dibenzylaminosuccinate (75 g) in dry tetrahydrofuran (THF) (400 ml) was added dropwise without heating over a period of about 1 hr, the heat of reaction causing a gentle refluxing. After additional heating for about 1.5 hr and stirring for a further 1.5 hr at room temperature, ethyl acetate (25 ml) was carefully added and the reaction mixture cooled to 0–5°. After successive cautious additions of water (25 ml) and 4 *N* NaOH (50 ml) the inorganic precipitate formed was removed by filtration and extracted (hot $CHCl_3$, 1 l.). The combined organic

(10) F. I. Carroll, *J. Org. Chem.*, **31**, 366 (1966).
(11) W. Wenner, *ibid.*, **13**, 26 (1948).

Formula	% calcd C	H	N	S	Hal	% found C	H	N	S	Hal
$C_{12}H_{24}N_2O_{10}S_2$	34.28	5.75	6.66	15.25		34.50	5.96	6.68	15.18	
						34.43	5.91	6.69	15.21	
$C_{10}H_{16}N_2O_4$	52.62	7.07	12.27			52.49	6.98	12.08		
						52.53	7.19	12.34		
$C_{18}H_{22}O_4$	71.50	7.33				71.47	7.41			
						71.31	7.23			
						71.32	7.36			
$C_{20}H_{26}O_8S_2$	52.38	5.72		13.99		52.46	5.79		13.91	
						52.30	5.70		13.86	
						52.43	5.74		13.88	
$C_{18}H_{24}N_2O_2\cdot 2HCl$	57.91	7.02	7.51		18.99	57.88	6.97	7.43		19.11
						57.75	7.14	7.39		19.10
						57.52	7.13	7.40		18.89
$C_{10}H_{20}N_2O_6$	45.44	7.63	10.60			44.86	7.66	10.66		
						45.21	7.71	10.64		
$C_{22}H_{28}N_2O_4$	68.73	7.34	7.29			68.65	7.33	7.25		
						68.73	7.54	7.15		
						68.90	7.57	7.35		
$C_4H_{12}N_2O_2\cdot 2HBr$	17.04	5.00	9.94		56.68	17.20	5.15	9.76		56.67
						16.78	5.29	9.62		56.39
						17.34	4.94	9.83		56.56
$C_4H_{12}N_2O_2\cdot 2HCl$	24.88	7.31	14.51		36.73	24.98	7.29	14.22		35.13
						25.01	7.56	14.38		36.82
						24.10	7.80			
$C_4H_{10}N_2O\cdot 2HBr$	18.20	4.58	10.62		60.55	18.06	4.67	10.50		60.58
						18.29	4.74	10.53		60.54
$C_8H_{20}N_2O_2\cdot 2HCl\cdot 2H_2O$[m]	33.69	9.19	9.82		24.86	33.97	9.13	9.67		24.94
[n]						33.80	9.17	9.63		25.04
$C_8H_{20}N_2O_2\cdot 2HCl\cdot H_2O$[o]	35.96	9.07	10.17		26.54	35.77	9.05	10.48		26.46

had mp 170–171° (MeOH). [k] This material was hygroscopic. [l] For material prepared by method K(a); for material prepared by method K(b) the value was −20.1°. [m] *Anal.* Calcd: H_2O, 12.6. Found: H_2O (Karl Fischer), 12.6. [n] *Anal.* Calcd: H_2O, 12.6. Found: H_2O (Karl Fischer), 12.6. [o] *Anal.* Calcd: H_2O, 6.7. Found: H_2O (Karl Fischer), 6.7.

extracts were evaporated under reduced pressure to give 43 g of crude product, mp 81–82.5°, sufficiently pure for further reaction. The **dihydrobromide** was prepared by addition of excess 48% HBr to a solution of the diamine in ethanol; mp 183.5–184.5° (ethanol).

Anal. Calcd for $C_{18}H_{24}N_2O_2\cdot 2HBr$: C, 46.77; H, 5.67; N, 6.06; Br, 34.58. Found: C, 46.73; H, 5.77; N, 6.28; Br, 34.46.

***meso*-1,4-Dichloro-2,3-dibenzylaminobutane Dihydrochloride.**—To a suspension of *meso*-2,3-dibenzylamino-1,4-butanediol (6.0 g) in ethanol (25 ml), the theoretical amount of ethanolic HCl was added and the resulting solution was evaporated under reduced pressure. To the residue $SOCl_2$ (60 ml) was added rapidly while stirring at 0–5°, and the mixture was stirred at room temperature for about 20 hr, followed by heating at 50–60° for 3 hr. Diethyl ether (150 ml) was added and the precipitate was washed with diethyl ether yielding 3.3 g of material. Trituration with ethanol (20 ml) gave 3.0 g of product, mp 168–176° dec.

Anal. Calcd for $C_{18}H_{22}Cl_2N_2\cdot 2HCl$: C, 52.70; H, 5.90; Cl, 34.57; N, 6.83. Found: C, 52.33; H, 5.97; Cl, 34.54; N, 6.65.

2,3-Bis(dimethylamino)-1,4-butanediol Dihydrochlorides (19·2HCl). Method J (Table II).—A solution of **12**·2HCl (5.8 g) and 35% formalin solution (12.0 g) in water (20 ml) was hydrogenated in the presence of 10% Pd–C (3.0 g) at 1.1 atm. The hydrogen uptake was complete in about 20 hr. The catalyst was removed by filtration and the resulting solution was evaporated under reduced pressure. Trituration with ethanol gave about 5 g of crude material.

Cyclic Sulfite of DL-2,3-Bis(dimethylamino)-1,4-butanediol Dihydrochloride.—A suspension of DL-2,3-bis(dimethylamino)-1,4-butanediol dihydrochloride dihydrate (0.5 g) in $SOCl_2$ (10 ml) was refluxed for about 20 hr. The resulting precipitate was washed with diethyl ether to give 0.5 g of material with mp 144–148° dec. Recrystallization from water–ethanol raised the melting point to 150–154° dec.[12]

(12) The melting point depended on the rate of heating which for the value stated was about 1°/min.

Anal. Calcd for $C_8H_{18}N_2O_3S\cdot 2HCl$: C, 32.54; H, 6.83; N, 9.49; S, 10.86; Cl, 24.02. Found: C, 32.53; H, 6.99; N, 9.53; S, 10.85; Cl, 23.97.

3,4-Diaminotetrahydrofuran Dihydrobromide (13·2HBr). Method K (Table II). (**a**)—A solution of **12**·2HCl (2.0 g) in 48% HBr (40 ml) was refluxed for about 20 hr and then evaporated under reduced pressure. Trituration with acetone gave about 2.5 g of crude **13**·2HBr.

(**b**)—A solution of (*S*:*S*)-**1** (1.0 g) in methanesulfonic acid (3.0 ml) and water (0.3 ml) was heated on a steam bath for about 2 hr and then refluxed for 30 min. Diethyl ether was added and the precipitate was triturated with a small amount of ethanol to give 0.35 g of (*S*:*S*)-**13**·$2CH_3SO_3H$.

Anal. Calcd for $C_4H_{10}N_2O_2\cdot 2CH_3SO_3H$: C, 23.22; H, 5.85; N, 9.03; S, 20.66. Found: C, 22.95; H, 6.17; N, 8.98; S, 21.35.

This material (0.2 g) was converted to the **dihydrobromide** by crystallization from 3.0 ml of 48% HBr, to give 0.18 g of (*S*:*S*)-**13**·2HBr. The infrared spectrum (KBr) and the analysis were identical with those of the material prepared as in part a.

(2*S*:3*S*)-1,4-N-2,3-O-Tetracarbethoxy-1,4-diamino-2,3-butanediol [(*S*:*S*)-16].—To a suspension of (*S*:*S*)-**15**·2HBr[l] (28.2 g) in pyridine (200 ml), ethyl chloroformate (50 ml) was added dropwise over a period of about 30 min while stirring at 0–5°. After additional stirring for about 1.5 hr the mixture was poured into ice–2 *N* HCl (1 l.). The separated oil was extracted with $CHCl_3$, and the extract, after drying ($MgSO_4$), was evaporated under reduced pressure. Trituration with petroleum ether (bp 50–70°) gave 28.7 g of crude (*S*:*S*)-**16**, mp 82.5–84°. After recrystallization from diethyl ether–petroleum ether the melting point was unchanged, $[\alpha]^{20}D$ −43.2° (*c* 2, ethanol).

Anal. Calcd for $C_{16}H_{28}N_2O_{10}$: C, 47.05; H, 6.91; N, 6.86. Found: C, 46.84; H, 6.98; N, 6.90.

(2*S*:3*S*)-N,N′-Dicarbethoxy-1,4-diamino-2,3-butanediol [(*S*:*S*)-17] and (5*S*:α*S*)-5-(β-Carbethoxyamino-α-hydroxyethyl)-oxazolidone (2) [(*S*:*S*)-18].—A solution of (*S*:*S*)-**16** (10.2 g) and KOH (3.0 g) in ethanol (50 ml) was stirred at room temperature for about 1.5 hr. After acidification with 10 *N* ethanolic HCl

(5 ml) the inorganic precipitate was removed by filtration (Dicalite), and the resulting clear solution was evaporated under reduced pressure. The residue was extracted with acetone (75 ml) which after treatment with decolorizing carbon was removed under reduced pressure. The residue was extracted with diethyl ether (50 ml) from which (*S*:*S*)-**17** (0.6 g) separated when kept at room temperature overnight. The melting point was 112–113.5°, $[\alpha]^{20}D$ +21.2° (*c* 2, ethanol).

Anal. Calcd for $C_{10}H_{20}N_2O_6$: C, 45.44; H, 7.63; N, 10.60. Found: C, 45.20; H, 7.84; N, 10.50.

The material (3.2 g) which remained undissolved after the ether extraction was recrystallized from acetone to give 1.4 g of (*S*:*S*)-**18**, mp 141–143°, $[\alpha]^{20}D$ +39.3° (*c* 2, ethanol).

Anal. Calcd for $C_8H_{14}N_2O_5$: C, 44.03; H, 6.47; N, 12.84. Found: C, 43.86; H, 6.66; N, 12.91.

(2*S*:3*S*)-N,N′-Dicarbethoxy-1,4-diamino-2,3-butanediol 2,3-Bismethanesulfonate [(*S*:*S*)-2]. (a)—To a suspension of (*S*:*S*)-**14**·$2CH_3SO_3H$[1] (9.4 g) in pyridine (75 ml), ethyl chloroformate (9.5 ml) was added dropwise over a period of about 40 min while stirring at 0–5°. After additional stirring for about 30 min the mixture was poured into ice–2.5 *N* HCl (300 ml). The precipitate was washed with water and dried *in vacuo* yielding crude (*S*:*S*)-**2** (7.3 g). After recrystallization from tetrachloroethane the melting point was 162.5–163.5°, $[\alpha]^{20}D$ −12.6° (*c* 2, DMF).

(b)—To a solution of (*S*:*S*)-**17** (0.3 g) in pyridine (3 ml), methanesulfonyl chloride (0.3 ml) was added dropwise over a period of about 20 min while stirring at 0–5°. After additional stirring for about 30 min the mixture was poured into ice–2.5 *N* HCl (12 ml). The precipitate was washed with water and dried *in vacuo* yielding crude (*S*:*S*)-**2** (0.45 g), melting at 155–157°. Recrystallization from tetrachloroethane raised the melting point to 162–163°, $[\alpha]^{20}D$ −13.7° (*c* 2, DMF). The infrared spectrum (KBr) and the analysis were identical with those of the material prepared as in part a.

Anal. Found: C, 34.35; H, 5.70; N, 6.55; S, 15.22.

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Syntheses of Metabolites of 7,12-Dimethylbenz[*a*]anthracene. 4-Hydroxy-7,12-dimethylbenz[*a*]anthracene, 7-Hydroxymethyl-12-methylbenz[*a*]anthracene, Their Methyl Ethers, and Acetoxy Derivatives[1a]

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The synthesis of 4-hydroxy-7,12-dimethylbenz[*a*]anthracene (V) was accomplished by a scheme starting with the preparation of known 4-methoxybenz[*a*]anthracene (I). This was oxidized to 4-methoxybenz[*a*]anthraquinone (II) which was treated with methylmagnesium iodide and the Grignard complex slowly added to HI in methanol affording 4-methoxy-7-iodomethyl-12-methylbenz[*a*]anthracene (III). This was reduced to 4-methoxy-7,12-dimethylbenz[*a*]anthracene (IV) which was cleaved with HBr–AcOH to give 4-hydroxy-7,12-dimethylbenz[*a*]anthracene (V). 7-Hydroxymethyl-12-methylbenz[*a*]anthracene (X) was synthesized from 7-iodomethyl-12-methylbenz[*a*]anthracene (IX). This was conveniently prepared from benz[*a*]anthraquinone (VII) by the Grignard method. A dioxane solution of IX was converted to the corresponding hydroxy compound. 4-Hydroxy-7,12-dimethylbenz[*a*]anthracene, 7-hydroxymethyl-12-methylbenz[*a*]anthracene, and 7,12-dihydroxymethylbenz[*a*]anthracene were identified in rat liver homogenates as metabolites of 7,12-dimethylbenz[*a*]anthracene. Preliminary data on carcinogenic activity of these metabolites are presented.

Although many chemical carcinogens are believed to exert their remarkable effects only after being transformed to active forms *in vivo*, in the case of polycyclic aromatic hydrocarbons it is not clear whether the parent compound or one or more metabolites are responsible for producing cancers in test animals. Since polycyclic aromatic hydrocarbons are, according to present evidence,[2a] metabolized to hydroxy derivatives by rats, it is conceivable that the active forms of the hydrocarbon are hydroxylated metabolic products.[2b,c] The synthesis of hydroxylated derivatives of the potent carcinogen 7,12-dimethylbenz[*a*]anthracene (DMBA) and related compounds was therefore undertaken to provide authentic metabolites for carcinogenic testing and for direct comparison with compounds isolated in animal studies. This paper is the first of a series describing the synthesis and biological properties of metabolites of polycyclic hydrocarbons.

Initial effort was directed toward the synthesis of 4-hydroxy-7,12-dimethylbenz[*a*]anthracene which was earlier identified as a metabolite of DMBA by its fluorescence spectrum after conversion to the methoxy derivative.[3] The sequence of reactions is outlined in Scheme I. The 4-methoxybenz[*a*]anthraquinone (II) required for this synthesis was prepared by oxidation of known 4-methoxybenz[*a*]anthracene (I). This compound was prepared from 4-hydroxybenz[*a*]anthracene[4a] by methylation with dimethyl sulfate according to Sempronj.[4b] 4-Methoxybenz[*a*]anthraquinone was condensed with methyl Grignard, the Grignard solution

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(2) (a) E. Boyland, *Brit. Med. Bull.*, **20**, 121 (1964). (b) Boyland[2a] has suggested epoxides as intermediates in the metabolism of polycyclic aromatic hydrocarbons. These are alkylating agents and would be expected to react with cellular constituents. (c) M. S. Newman and S. Blum, *J. Am. Chem. Soc.*, **86**, 5598 (1964), have shown that epoxides of aromatic hydrocarbons are sensitive to acid and are converted to hydroxy derivatives.

(3) (a) F. Dickens, *Brit. Empire Cancer Campaign*, 55 (1945). (b) After this synthesis was completed we learned (private communication) that the preparation of 4-methoxy-7,12-dimethylbenz[*a*]anthracene was accomplished by Professor Dickens using essentially the same scheme.

(4) (a) J. Cason and L. F. Fieser, *J. Am. Chem. Soc.*, **62**, 2681 (1940); (b) A. Sempronj, *Gazz. Chim. Ital.*, **69**, 448 (1939).