pressure to give 232 g of an oily product. It was fractionally distilled *in vacuo* and the fraction boiling at 85–95° (2.5 mm) was collected. Gc analysis indicated an over-all yield of 33% (87 g) of product. An analytical sample had bp 93° (2.5 mm), ir 6.5 μ (NO_2). *Anal.* ($C_5H_9NO_4$) C, H.

4-Nitrobutyl Acetate (X, n = 4).—To a stirred suspension of 270 g (1.75 moles) of $AgNO_2$ in 400 ml of anhydrous Et_2O was added dropwise, at 0°, 235 g (1.20 moles) of 4-bromobutyl acetate. After the addition was complete (*ca.* 3 hr), the reaction mixture, protected from moisture and light, was stirred at 0° for 24 hr and then at room temperature for an additional 48 hr. The resulting mixture was filtered and the solid (AgBr) was washed with two 80-ml portions of Et_2O. The combined filtrate and washings were evaporated under reduced pressure to yield 196 g of a yellow oil. It was fractionally distilled to give 84.9 g (44% yield) of product and 75.1 g (38% yield) of the nitrite ester. (The yield and purity were determined by ir and gas chromatography.) Repeated fractionation of the nitro ester yielded an analytically pure compound, bp 103–105° (2.2 mm). *Anal.* ($C_6H_{11}NO_4$) C, H, N.

5-Nitroamyl acetate (X, n = 5), bp 124–126° (2.5 mm), was prepared in a similar fashion, yield 42%.

2-Bromoethyl Acetate and Silver Nitrite.—Under reaction conditions similar to those above, only the nitrite ester (9.7 g, 72% yield) was obtained from 16.7 g of 2-bromoethyl acetate and 23.2 g of $AgNO_2$. The product showed an ir band at 6.1 μ (ONO), and did not condense with an aromatic aldehyde. *Anal.* ($C_4H_7NO_4$) C, H, N.

General Procedure for the Condensation of Aromatic Aldehyde and ω-Nitro-ψ-alkan-1-ol Acetate.—A mixture of 0.1 mole of the appropriate aromatic aldehyde, 0.1 mole of 4-nitrobutyl acetate, and 0.12 mole of NH_4OAc in 140 ml of glacial AcOH was refluxed for 2 hr. The solvent was removed under reduced pressure and the residue was poured into 500 ml of ice–H_2O. The mixture was then extracted with four 200-ml portions of Et_2O. The combined ethereal extracts were washed with three 80-ml portions of H_2O and dried (Na_2SO_4). The solvent was then removed and the oily residue was triturated with a small amount of CH_3OH. On standing (or freezing in a Dry Ice–acetone bath) the product slowly separated as a yellow solid. It was filtered off and recrystallized from MeOH (see Table I).

2,2,2′,2′-Tetraphenyldivinylamine.—A mixture of 90.6 g (0.1 mole) of diphenylacetaldehyde, 24.2 g (0.15 mole) of 4-nitrobutyl acetate, 5.0 g (0.065 mole) of NH_4OAc in 300 ml of AcOH was refluxed for 2 hr. The reaction mixture was cooled. The resulting white solid was isolated by filtration, washed with two 10-ml portions of AcOH followed by two 10-ml portions of petroleum ether (bp 35–60°) to give 8.5 g (46% yield) of product, mp 145–147°, $\lambda_{max}^{C_2H_5OH}$ 358 μ (log ϵ 4.40). No ir C=O absorption band was noted. This product was identical with that prepared by dehydration of 2-hydroxy-2,2-diphenylethylamine[21] or by chemical reduction of 1,1-diphenyl-2-nitroethene.[22] The same product was obtained in quantitative yield by refluxing a mixture of 19.6 g of diphenylacetaldehyde and 11.6 g of NH_4OAc in 150 ml of AcOH.

1-Nitro-2-(9-phenanthryl)ethene.—The following procedure is a modification of that reported by Mosettig and May.[24] To a warm (30–40°) solution of 10.3 g (0.050 mole) of 9-phenanthrenecarboxaldehyde and 3.1 g (0.05 mole) of $MeNO_2$ in 200 ml of EtOH was added in 5 min, with stirring, 50 ml of 8% aqueous KOH. The solution was stirred for another 30 min and subsequently poured into 160 ml of 15% HCl with cooling and vigorous stirring. The resulting yellow solid was filtered off and washed (H_2O) to give 11.7 g of crude product, mp 140°. It was recrystallized (EtOH) to give 7.0 g of pure product, mp 173–175° (lit.[24] mp 173–173.5°).

Reduction of 4-Nitro-5-(9-phenanthryl)-4-penten-1-ol Acetate (II).—To 200 ml of THF containing 16 g of $LiAlH_4$ was added dropwise a solution of 15.6 g (0.045 mole) of II in 120 ml of THF. After the addition was complete, the mixture was stirred at room temperature for 16 hr and then decomposed (H_2O). The solid was filtered and washed with two 50-ml portions of THF. The combined filtrate and washings were evaporated (temperature <40°) to dryness *in vacuo*. To the residue was added, with stirring, 200 ml of Et_2O and 200 ml of C_6H_6. After being refrigerated overnight the white solid (6.8 g, mp 75–140°), which consisted of a mixture of oxazine and saturated amino alcohol, was collected by filtration. Fractional recrystallization of the white solid from 500 ml of C_6H_6 gave 1.0 g (8% yield) of 3-(9-phenanthrylmethyl)-3,4,5,6-tetrahydro-1,2-oxazine, mp 187–189° (lit.[3] mp 185°). The mother liquor, after being concentrated to 50 ml and diluted with 150 ml of Et_2O, deposited 3.2 g of 4-amino-5-(9-phenanthryl)pentanol, mp 118–120° (lit.[3] mp 120°).

4-Formamido-5-(9-phenanthryl)penten-1-ol Formate.—A solution of 6.5 g (0.018 mole) of the amino alcohol, obtained from the aforementioned $LiAlH_4$ reduction, in 100 ml of HCOOAc was stirred for 16 hr at room temperature. Excess anhydride was removed *in vacuo* to an oily residue. The residue was triturated with 200 ml of anhydrous Et_2O for 10 min and the resulting white solid (3.3 g) was collected by filtration, mp 145–148°. Recrystallization of 0.5 g of the solid from 50 ml of C_6H_6 gave 0.4 g of pure product, mp 147–149° (lit.[3] mp 145°).

(24) E. Mosettig and E. L. May, *J. Am. Chem. Soc.*, **60**, 2962 (1938).

Potential Anticancer Agents. V. New Aromatic Nitrogen Mustards Related to 3-[N,N-Bis(2-chloroethyl)amino]-4-methylbenzoic Acid

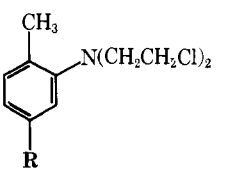
A. CAMBANIS, V. DOBRE, AND I. NICULESCU-DUVĂZ

Oncological Institute, Bucharest, Romania

Received July 8, 1968

The synthesis of new esters and amides of 3-[N,N-bis(2-chloroethyl)amino]-4-methylbenzoic acid is described. New nitrogen mustards, derivatives of phosphoric and sulfonic aromatic acids, are also reported. A quantitative relation between hydrolysis rate of these N-mustards and basicity of precursor amines was established. Enhancement of the hydrolysis rate due to the steric effect of *o*-methyl groups was pointed out. Antitumor activity was tested against Jensen sarcoma, Walker 256 carcinosarcoma, and Guérin T8 carcinoma.

Previous studies on the relationship between chemical reactivity and antitumor properties in aromatic nitrogen mustards series led us to assign some special "carrier" properties to the benzoic acid structure.[1] Promising pharmacological and clinical results[2] obtained with 3-[N,N-bis(2-chloroethyl)amino]-4-methylbenzoic acid (Ia, R = CO_2H) prompted a closer examination of compounds I, in which the chemical re-



I

activity of the nitrogen mustard moiety is enhanced by an *o*-methyl substituent.[3]

(1) O. Costăchel, I. Niculescu-Duvăz, A. Cambanis, and G. Ciustea, Proceedings of the National Conference of Oncology, Bucharest, Nov 4–6, 1965.

(2) O. Costăchel and I. Mogos, *Oncol. Radiol.*, **7**, 255 (1968).

(3) I. Niculescu-Duvăz, M. Ionescu, A. Cambanis, M. Vitan, and V. Feyns, *J. Med. Chem.*, **11**, 500 (1968).

The following additional members of the series were prepared in order to study the biological effect of insertion of other strong electron-attracting groups (keeping the chemical reactivity of the nitrogen mustard function at about the same level), as well as the effect of varying the type of acid function, on the carcinostatic activity: (1) compounds without acidic character, and (2) compounds with other acid functions (R = SO_3H, PO_3H_2). In the first group falls functional derivatives of Ia (esters and amides) whose hydrolysis rates (k_{66}) differ rather slightly from that of compound Ia and derivatives Im, o, p with lower "biological compatibility" (except compound Io, R = CHO, which by *in vivo* oxidation may lead to Ia). Mention should be made that an isomer of Io, namely, 4-[*N*,*N*-bis(2-chloroethyl)amino]-3-methylbenzaldehyde, had already been prepared and appears to be more active than *p*-aminobenzaldehyde nitrogen mustard.[4]

Synthesis.—The new nitrogen mustards are summarized in Table I. Synthesis of the functional derivatives of Ia was carried out *via* acid chloride II (Scheme I), as well as *via* amines IV (See Table II), prepared in order to perform basicity measurements. Confirmation of the structure assigned to esters Ib–d was provided by their acid hydrolysis which led in all cases to near-quantitative yields of Ia.

Aromatic nitrogen mustards with sulfonic or sulfonamidic groups have been only slightly investigated.[5] The synthesis of compounds II and Im, was not accomplished by direct chlorosulfonation[5a] in order to avoid isomer formation but by introduction of the N-mustard moiety on 4-methylbenzenesulfonic acid or amide (Scheme II).

Protection of the sulfonic acid group is done more conveniently by means of the methyl ester (although slightly hydrolyzable) than by the respective sulfonamide whose hydrolysis requires conditions too drastic to preserve the nitrogen mustard group.

There is a paucity of information concerning the synthesis and activity of N-mustards derived from benzenephosphonic acids.[6] Introduction of the phosphonic group (as dimethyl ester) into toluene was

TABLE I

NEW AROMATIC NITROGEN MUSTARDS (I)

Compd	R	Formula[a]	Mp, °C	Hydrolysis rate[b] %	Hydrolysis rate[b] − Log K_{66}
Ib	CO_2CH_3	$C_{13}H_{17}Cl_2NO_2$	Oil[c]	52.0	3.39
Ic	$CO_2CH(CH_3)_2$	$C_{15}H_{21}Cl_2NO_2$	45	52.8	3.38
Id	$CO_2C_{17}H_{35}$[d]	$C_{29}H_{49}Cl_2NO_2$	15[e]		
Ie	$CO_2CH_2CH_2N(C_2H_5)_2HCl$[f]	$C_{18}H_{29}Cl_3N_2O_2$	109		
If	$CONH_2$[g]	$C_{12}H_{16}Cl_2N_2O$	160–162	71.4	3.13
Ig	$CONHC_2H_5$[h]	$C_{14}H_{20}Cl_2H_2O$	82–83	68.6	3.19
Ih	CON⟨H,O⟩[i]	$C_{14}H_{20}Cl_2N_2O_2$	90–91	81.4	3.03
Ii	CON⟨H⟩[j]	$C_{17}H_{24}Cl_2N_2O$	49–50	82.2	3.02
Ij	CON⟨H,O⟩	$C_{16}H_{22}Cl_2N_2O_2$	123–126		
Ik	CONH–⟨N,S⟩[k]	$C_{15}H_{17}Cl_2N_3OS$	118–119	52.1	3.39
Il	SO_3H	$C_{11}H_{15}Cl_2NO_3S$	223–226		
Im	SO_2N⟨H,O⟩	$C_{15}H_{22}Cl_2N_2O_3S$	85–87		
In	PO_3H_2	$C_{11}H_{16}Cl_2NO_3P$	[l]	41.8	3.52
Io	CHO[f]	$C_{12}H_{15}Cl_2NO$	103–105	56.2	3.32
Ip	NO_2[g]	$C_{11}H_{14}Cl_2N_2O$	68–69	14.0	4.06

[a] All compounds were analyzed for C, H, N, Cl. [b] Determined according to Ross[11] after 0.5 hr, in H_2O–Me_2CO (1:1) with potentiometric titration of Cl^-. [c] Prepared from n-$C_{17}H_{35}OH$. [d] Recrystallized from C_6H_6–petroleum ether (bp 60–90°). [e] Recrystallized from C_6H_6. [f] Recrystallized from petroleum ether (bp 90–120°). [g] Recrystallized from toluene–petroleum ether (bp 90–120°). [h] Recrystallized from EtOAc. [i] Recrystallized from MeOH. [j] $n^{25}D$ 1.5474. [k] $n^{25}D$ 1.5010. [l] No melting till 350°.

SCHEME I

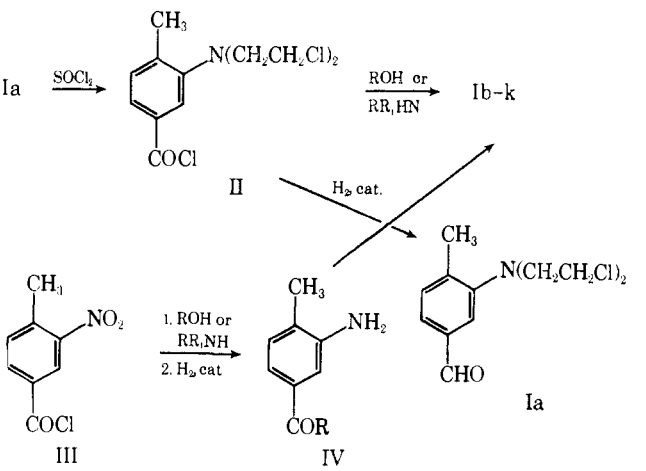


TABLE II

CHARACTERISTICS OF INTERMEDIATE AMINES

Compd[a]	R	Mp, °C	Basicity,[b] pK_a
IVb	CO_2CH_3	116	3.34 ± 0.02[c]
IVc	$CO_2CH(CH_3)_2$	66	3.35 ± 0.03
IVd	$CO_2C_{17}H_{35}$	68	
IVf	$CONH_2$	123–125	3.52 ± 0.02[d]
IVg	$CONHC_2H_5 \cdot HCl$	186–190	3.55 ± 0.05
IVh	CON⟨	Oil	3.48 ± 0.03
IVi	CON⟨H⟩	127–128	3.40 ± 0.03
IVj	CON⟨H,O⟩	153–154	3.23 ± 0.03
IVk	CONH–⟨N,S⟩	114–114.5	3.10 ± 0.03
VIII	SO_2OCH_3	63–65	1.86 ± 0.02
VIIIm	SO_2N⟨H,O⟩	160–161	2.62 ± 0.04
VIIIn	$PO(OCH_3)_2$	Oil	2.90 ± 0.04
VIIIp	NO_2	103–105[e]	2.32 ± 0.03[f]

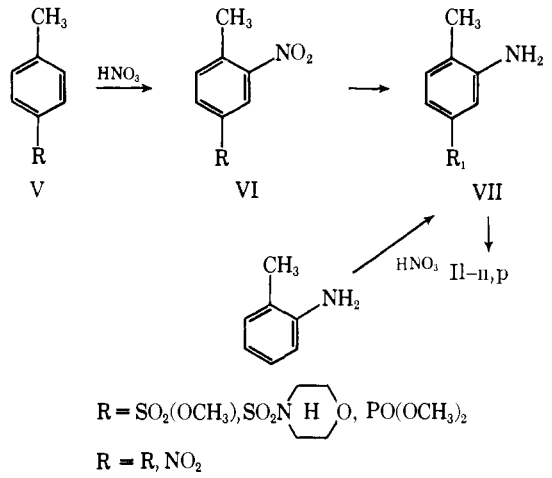
[a] All compounds were analyzed for N. [b] Determined spectrophotometrically. [c] pK_a = 3.39, calculated according to J. Clark and D. Perrin's [*Quart. Rev.* (London), **28**, 295 (1964)] relation ($pK_a = 4.58 - 2.81\Sigma\sigma$) for a value of σ_n = 0.32. [d] pK_a = 3.50 for σ_m = 0.28. [e] F. Ullman and E. Grether, *Ber.*, **35**, 337 (1902), give mp 105–107° (EtOH). [f] pK_a = 2.29 for σ_m = 0.71.

(4) (a) F. D. Popp, *J. Org. Chem.*, **26**, 1566 (1961); (b) *J. Med. Chem.*, **7**, 210 (1964).

(5) (a) M. H. Benn, A. M. Creighton, B. J. Johnson, N. L. Owen, and G. R. White, *J. Chem. Soc.*, 3395 (1964); (b) P. J. Barnard, G. F. Danielli, R. Hawkins P. Hebbron, A. Muggleton, D. Triggle, and M. Triggle, *Archiv. Ital. Patol. Clin., Tumori*, **8**, 142 (1965).

(6) F. Kagan, R. D. Birkenmayer, and R. F. Sturbe, *J. Am. Chem. Soc.*, **81**, 3026 (1959).

SCHEME II



achieved by direct phosphonation[7] where, besides the mixture of *ortho*, *meta*, and *para* isomers quoted in the literature, we also obtained a diphosphonated product, which is perhaps tetramethyl methylbenzene-2,4-diphosphonate (according to the analysis of the nitro compound). The *para* isomer was separated and purified *via* the free acid. It was reesterified with diazomethane[6] and converted by nitration[8] to VI (R = $PO(OCH_3)_2$), identified by the uv spectrum[9] of the free acid VI (R = PO_3H_2). The melting point of this compound is in contrast with the above paper[7] but agrees with earlier data.[10]

Final acid hydrolysis (in concentrated HCl) led to a mixture of monoester and free acid In, which was then separated by passage on an ion-exchange resin column.

Physical–Chemical Data.—Hydrolysis rates of the new N-mustards were determined according to the standard procedure[11] and rate constants were calculated on the hypothesis of unimolecular kinetics (see Table I). Basicity of precursor amines was measured spectrophotometrically (see Table II). Correlation of these parameters for Ia–p leads to the following equation (see Figure 1)

$$\log k_{66} = -5.58 + 0.678 pK_a \qquad (1)$$

where the steric effect of the *o*-methyl group resulted in a significant enhancement of the N-mustard moiety hydrolysis rate.[3]

Pharmacological Data.—The 15 nitrogen mustards were tested against Jensen sarcoma, Walker 256 carcinosarcoma, and Guérin T8 carcinoma. In all cases, treatment began 7 days after tumor transplantation and lasted 14 days. Doses ($0.2–0.1LD_{50}$) were administered daily.[12] Inhibition of tumor growth was calculated according to standard formula (Table III).

Experimental Section

Ir spectra were determined on a UR 10 Zeiss Jena spectrophotometer in KBr disks or in solution (CCl_4). N-Mustards Ib–p were characterized by stretching vibrations of C–Cl bond at 670–658 cm^{-1} for P_H and 760–730 cm^{-1} for P_C configurations (lit.[13] 648 and 730 cm^{-1}). Absorption bands of functional groups in structure I (esters, amides, etc.) were as expected.

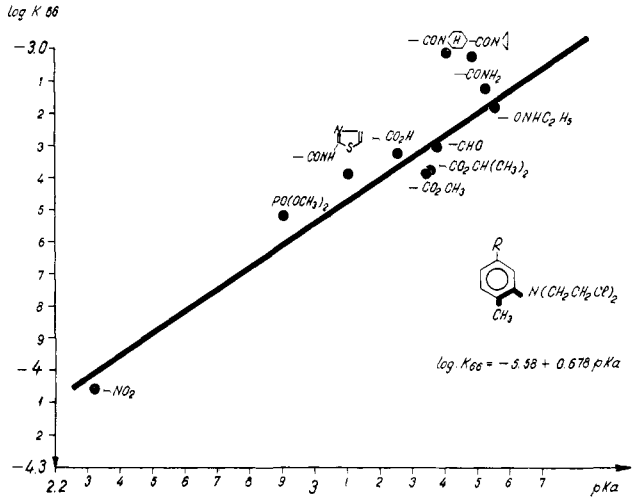


Figure 1.

TABLE III

BIOLOGICAL DATA OF NEW NITROGEN MUSTARDS[a]

Compound	LD_{50}, mg/kg (mouse)	Inhib, %[b] Jensen sarcoma	Walker 256 carcinosarcoma	Guérin T8 carcinosarcoma
Ib	20	97	84	
Ic	20	97		72
Id	300	28		
Ie	20	90	67	49
If	14	86	72	
Ig	11.5	96	87	77
Ih	16.6	95	80	
Ii	50	93	56	
Ij	16.6	83		76
Ik	50	82		48
Il	200	53		
Im	72	80	40	
In	72	47	33	
Io	20	74		
Ip	250	90		26

[a] Screening was carried out by Dr. V. Dobre. [b] Doses were between 0.2- and $0.1LD_{50}$.

3-[N,N-Bis(2-chloroethyl)amino]-4-methylbenzoyl chloride (II) was prepared from the free acid using $SOCl_2$ oil, yield 70%. *Anal.* ($C_{12}H_{14}Cl_3NO$) C, H, N.

Substituted Amides (Ig–k).—To a stirred solution of 0.01 mole of II in 50 ml of dry PhH, 0.02 mole of corresponding amines was added, at 20°. After standing overnight, the separated solid was removed by filtration. The filtrate was treated with charcoal and evaporated to dryness under vacuum. The residue was recrystallized from the proper solvent or purified by alumina column chromatography.

The **esters (Ib–e)** were prepared from II by classical procedures.

3-[N,N-Bis(2-chloroethyl)amino]-4-methylbenzamide (If).—A solution of 4 g (0.015 mole) of II in 15 ml of dioxane was added to a stirred mixture of 20 ml of 15% NH_3 and 100 ml of 10% aqueous NH_4Cl. The separated oily substance (solidifying upon stirring) was taken up in C_6H_6 (five 200-ml portions). The dried (Na_2SO_4) and filtered solution was evaporated *in vacuo* to give a white solid residue. Recrystallization from dry C_6H_6 gave 1.9 g (51%) of amide.

3-[N,N-Bis(2-chloroethyl)amino]-4-methylbenzaldehyde (Io).—Through a boiling solution of 5 g (0.019 mole) of II in 250 ml of toluene containing 4 g of 5% Pd–$CaCO_3$, a stream of H_2 was passed for 1–2 hr, until the theoretical amount was absorbed (titration of evolved HCl). After filtration of the catalyst and removal of the solvent under reduced pressure, 3.5 g (80%) of a

(7) (a) G. M. Kossolapoff, *J. Am. Chem. Soc.*, **69**, 2020 (1947); (b) *ibid.*, **74**, 4119 (1952).
(8) G. M. Kossolapoff, *ibid.*, **71**, 1876 (1949).
(9) L. D. Freedman and G. O. Doak, *J. Org. Chem.*, **26**, 2082 (1961).
(10) A. Michaelis, *Ann.*, **293**, 270 (1896).
(11) W. C. J. Ross, *J. Chem. Soc.*, 183 (1949).
(12) V. Dobre and G. Maltezeanu, personal communication.
(13) S. J. Shippman, V. L. Flott, and S. Krimm, *Spectrochim. Acta*, **18**, 1613 (1962).

viscous oil was obtained; it crystallized on trituration with petroleum ether (bp 90–120°); dinitrophenylhydrazone, from EtOH, mp 187–189°. *Anal.* ($C_{18}H_{16}Cl_2N_5O_4$) N.

Dimethyl 4-methylbenzenephosphonate [V, R = $PO(OCH_3)_2$] was obtained according to Kossolapoff,[7a] bp 143–146° (2–3 mm), $n^{20}D$ 1.5060, yield 81%. A side product was also obtained, bp 180° (2–3 mm), $n^{20}D$ 1.5470, yield 16%, that remains as a residue during distillation of the reaction mixture and which is assumed (after analysis of the nitrated product) to be tetramethyl methylbenzene-2,4-diphosphonate.

V [R = $PO(OCH_3)_2$] (2 g, 0.01 mole) in 20 ml of HCl (*d* 1.19) was refluxed for 7 hr and gave after 24 hr of standing at room temperature 1.1 g (65%) of 4-methylbenzenephosphonic acid, mp 189–190° (H_2O).[10]

Dimethyl 3-Nitro-4-methylbenzenephosphonate [VI, R = $PO(OCH_3)_2$].—A mixture of 5.2 ml of HNO_3 (*d* 1.42) and 6 ml of H_2SO_4 (*d* 1.83) was added slowly to a cooled and stirred solution (0–5°) of 16 g (0.08 mole) of V [R = $PO(OCH_3)_2$] in 60 ml of H_2SO_4 (*d* 1.83). After complete addition, the mixture was stirred for an additional 0.5 hr and poured over 300 g of crushed ice. The resulting oil was extracted (C_6H_6), washed (H_2O), dried (Na_2SO_4), and evaporated to dryness, giving 16.5 g (84%) of VI as an oil, bp 170° (2–3 mm), $n^{20}D$ 1.5330. *Anal.* ($C_9H_{12}NO_5P$) N.

The following compounds were obtained similarly: **methyl 3-nitro-4-methylbenzenesulfonate,** mp 52–53° (C_6H_6–petroleum ether, 1:1), yield 98% [*Anal.* ($C_7H_7O_5S$) N]; **3-nitro-4-methylbenzenesulfonyl morpholide,** mp 119–120° [*Anal.* ($C_{11}H_{14}N_2O_5S$)N].

VI (2 g, 0.008 mole) in 20 ml of HCl (*d* 1.19) was refluxed for 7 hr and gave, after 2 days of standing at room temperature, 0.9 g (53%) of 3-nitro-4-methylbenzenephosphonic acid: mp 189° (EtOAc);[10] uv spectrum, λ_{max} 256 mμ (ε 4265).

Dimethyl 3-Amino-4-methylbenzenephosphonate [VII, R = $PO(OCH_3)_2$].—VI [R = $PO(OCH_3)_2$] (10 g, 0.04 mole) in 200 ml of anhydrous MeOH was hydrogenated at atmospheric pressure and room temperature with 6 g of 5% Pd–$CaCO_3$ catalyst. The theoretical amount of H_2 was absorbed in 0.3 hr. After catalyst removal and solvent evaporation under reduced pressure, the oily residue thus obtained was taken up in dry C_6H_6, treated with charcoal, and evaporated to dryness giving 6.5 g (74%) of VII [R = $PO(OCH_3)_2$] as an oil: $n^{20}D$ 1.5490; uv spectrum, λ_{max} 305 mμ (ε 2340).

Similarly prepared were **3-amino-4-methylbenzenesulfonyl morpholide** (VIIm, Table II) and **methyl 3-amino-4-methylbenzenesulfonate** (VIII, Table II).

Hydroxyethylation was carried out according to the previously described method.[2] The following compounds were obtained (melting point, yield, N analysis): 3-[N,N-bis(2-hydroxyethyl)amino]-4-methylbenzenesulfonyl morpholide (oil, 86%, ($C_{15}H_{26}N_2O_6S$) N), 3-[N,N-bis(2-hydroxyethyl)amino]-4-methylbenzenesulfonic acid (150–152°, 90%, ($C_{11}H_{17}NO_5S$) N), dimethyl 3-[N,N-bis(2-hydroxyethyl)amino]-4-methylbenzenephosphonate (oil, $n^{20}D$ 1.5244), 93%, ($C_{13}H_{22}NO_5P$) N), and 3-[N,N-bis(2-hydroxyethyl)amino]-4-methylnitrobenzene (112–113° (toluene), 80%, ($C_{11}H_{16}N_2O_4$) N). Methyl 3-[N,N-bis(2-hydroxyethyl)amino]-4-methylbenzenesulfonate was not isolated, being directly subjected to chlorination.

Chlorination was accomplished as previously reported.[2] During chlorination of sulfonic derivatives methyl ester hydrolysis occured, giving directly II (R = SO_3H).

3-[N,N-Bis(2-chloroethyl)amino]-4-methylbenzenephosphonic Acid (In).—To 5 g (0.016 mole) of the corresponding bis(hydroxyethyl) derivative in 75 ml of dry C_6H_6 was added 8 ml of freshly distilled $SOCl_2$, and the mixture was refluxed for 2 hr. After solvent and excess $SOCl_2$ removal under vacuum, 30 ml of HCl (*d* 1.19) was added, and the solution was heated to boiling for 0.5 hr, treated with charcoal, and filtered to remove the resins. To the filtrate, 25 ml of HCl (*d* 1.19) was added, and the solution was refluxed for 10 hr and allowed to stand 2 days at room temperature. HCl removal under vacuum gave 4 g of a yellow oily residue consisting of a mixture of In and monomethyl ester of In.

Separation of the two compounds was done by dissolving the residue in H_2O, filtering the insoluble ester, and concentrating the solution after adding few drops of HCl; or, the residue was dissolved in the minimum amount of H_2O–EtOH (1:1) and passed over Merck 2 (weak basic) ion-exchange resin, which retained only the free acid In. Its elution was carried out with H_2O–EtOH–HCl (*d* 1.19), 9:9:1. Analysis of the solid (no melting point till 350°) was in agreement with the calculated data for In; uv spectrum, λ_{max} 257 mμ (ε 3454).

Acknowledgments.—We want to particularly thank Mrs. A. Serban and Mrs. G. Botez who carried out physical-chemical determinations, Mr. R. Homescu for the ir spectra, Mr. V. Feyns for the technical review of the manuscript, and Mr. I. Dărăbăneanu for technical assistance.

Immunosuppressive Activity of 2′-(3-Dimethylaminopropylthio)cinnamanilide (Cinanserin) and Related Compounds. IV[1]

JOHN KRAPCHO, ROBERT C. MILLONIG, CHESTER F. TURK, AND BLANCHE J. AMREIN

The Squibb Institute for Medical Research, New Brunswick, New Jersey 08903

Received July 19, 1968

Several test procedures have previously shown cinanserin, a potent inhibitor of serotonin, to be more active than azathioprine as an immunosuppressive agent. Seventy-two compounds related to cinanserin were tested for immunosuppressive activity using the mouse–sheep red blood cell procedure and compared with their antiserotonin activity. The syntheses and physical properties of the new analogs of cinanserin are also reported. Although most compounds of this series showed a similar degree of immunosuppressive and antiserotonin activities, several members exhibited a marked separation of these responses. Five compounds showing high-immunosuppressive low-antiserotonin activities are presently undergoing further biological evaluation.

Cinanserin,[2] a potent serotonin inhibitor, suppresses the primary immune response of mice to sheep red blood cells.[3] Subsequent studies showed cinanserin to be more active than azathioprine in suppressing the uptake of C^{14}-labeled leucine and thymidine by human lymphocytes stimulated by phytohemagglutinin and in prolonging the time of survival of skin grafts between congenic strains of mice differing at the H-2 locus.[4] Cinanserin also suppresses the secondary immune response of mice to sheep red blood cells and the develop-

(1) Previous paper: J. Krapcho and C. F. Turk, *J. Med. Chem.*, **9**, 809 (1966).

(2) Cinanserin is the approved generic name for 2′-(3-dimethylaminopropylthio)cinnamanilide.

(3) R. C. Millonig, B. J. Amrein, J. Kirschbaum, and A. Borman, *Proc. Soc. Exptl. Biol. Med.*, in press.

(4) G. H. Schwartz, E. Noblinder, R. R. Riggio, K. H. Stenzel, and A. L. Rubin, *Clin. Res.*, **16**, 323 (1968).