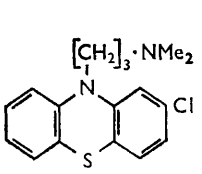
1007. *Synthesis of Potential Carcinostatic Derivatives of Benzo- and Dibenzo-phenothiazines.*

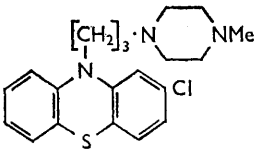
By David A. Shirley, John C. Gilmer, and W. D. Waters.

The syntheses of a number of derivatives of 12*H*-benzo[*a*]phenothiazine, 7*H*-benzo[*c*]phenothiazine, 14*H*-dibenzo[*a*,*h*]phenothiazine, 7*H*-dibenzo[*c*,*h*]-phenothiazine, and 14*H*-dibenzo[*a*,*c*]phenothiazine are reported. Particular attention is given to "nitrogen mustard" type derivatives containing $>N\cdot CH_2\cdot CH_2Cl$ and $>N\cdot CH_2\cdot CH_2\cdot N(CH_2\cdot CH_2Cl)_2$ groups because of their potential carcinostatic properties.

A number of phenothiazines, *e.g.*, chlorpromazine (I) and prochlorperazine (II), have anti-cancer activity. A wide variety of related compounds known as "nitrogen mustards" have now been examined for carcinostatic activity, and a significant number have found clinical application; they have in common the $ClCH_2\cdot CH_2\cdot N<$ and $(ClCH_2\cdot CH_2)_2N-$ groupings.



(I)



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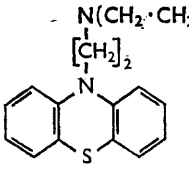

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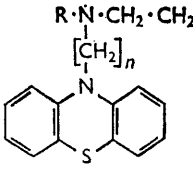
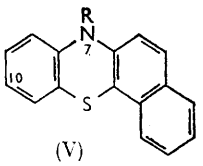


(IV)

The only prior record of nitrogen-mustard derivatives of phenothiazine and related types describes the synthesis of compound (III) [1] and eleven derivatives corresponding to compound (IV).[2]

This Paper reports the synthesis of nitrogen-mustard derivatives of several benzo- and dibenzo-phenothiazines. The method (see annexed scheme) is similar to that reported earlier,[1,3] utilizing 7*H*-benzo[*c*]phenothiazine (VI). The yields shown are typical of



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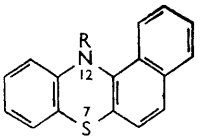

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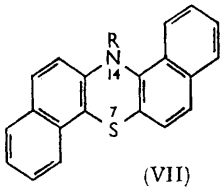
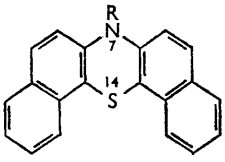
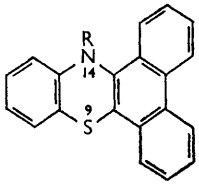


(VII)

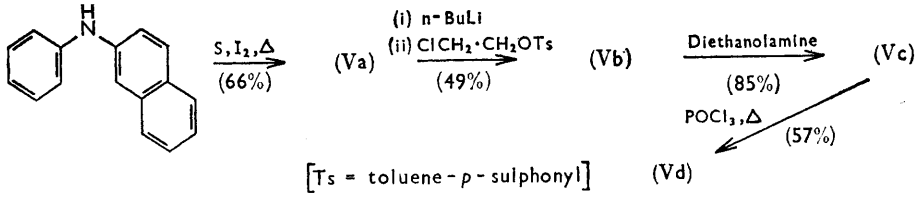
(VIII)





(IX)

a: R = H; b: R = $CH_2·CH_2Cl$; c: R = $[CH_2]_2·N(CH_2·CH_2OH)_2$; d: R = $[CH_2]_2·N(CH_2·CH_2Cl)_2$.



related reactions with the other phenothiazine types. This sequence was also used with benzo[*a*]phenothiazine (VIa), benzo[*c*]phenothiazine (Va), dibenzo[*a*,*h*]phenothiazine (VIIa), and dibenzo[*c*,*h*]phenothiazine (VIIIa).

Experimental

Melting points were determined on a " Mel-Temp " apparatus. Infrared spectra are for KBr discs, using a Perkin-Elmer Infracord instrument.

7-(2-*Chlorethyl*)-7H-*benzo*[c]*phenothiazine* (Vb).—n-Butyl-lithium [28 ml; 1·48M in pentane–heptane solvent (Foote Mineral Co.)] was added during 20 min. to a suspension of 7*H*-benzo-[*c*]phenothiazine [4] (10·0 g.) in anhydrous ether (700 ml.). The mixture was stirred for 45 min., cooled to 0°, a solution of 2-chloroethyl toluene-*p*-sulphonate (11·0 g.) in 50 ml. of ether added (10 min.), and stirred for 45 min. at 0° and for 20 hr. at 30°. Excess of benzene and water was added and the organic layer separated and concentrated to a small volume. The residual oil

[1] D. A. Shirley, K. Sen, and J. C. Gilmer, *J. Org. Chem.*, 1961, **26**, 3587.
[2] Societe des usines chemiques Rhone-Poulenc, Fr. P 1,167,514/1958.
[3] K. Sen and D. A. Shirley, *J. Org. Chem.*, 1961, **26**, 3861.
[4] D. A. Shirley and W. E. Tatum, *J. Amer. Chem. Soc.*, 1959, **81**, 496.

was dissolved in light petroleum (b. p. 100—115°)–benzene (7 : 3) and chromatographed on a column of Alcoa (F-20) alumina (3·0 × 40 cm.). The same solvent eluted the *product* (6·12 g., 49%), bright yellow crystals, m. p. 151—152° (Found: C, 69·7; H, 4·6; N, 4·4. $C_{18}H_{14}ClNS$ requires C, 69·3; H, 4·5; N, 4·5%).

7-[2-(*Bis*-2-*hydroxyethylamino*)*ethyl*]*benzo*[c]*phenothiazine* (Vc).—A mixture of the above chloroethyl compound (7·26 g.) and diethanolamine (100 ml.) was stirred for 60 hr. at 135—140°. Addition of water to the cooled mixture gave a suspension which was extracted several times with benzene. The extracts were evaporated to small volume and chromatographed on a column of Florisil (3·0 × 45 cm.). Benzene eluted a small quantity of unchanged 7-(2-chloroethyl)benzo[*c*]phenothiazine, and acetone–benzene (1 : 9) eluted the product, a viscous, yellow oil, formed in nearly quantitative yield.

The *hydrochloride* was prepared in 85% yield, m. p. 213·5—214·5° (from ethanol–ether) (Found: C, 63·3; H, 5·85; N, 6·6. $C_{22}H_{25}ClN_2O_2S$ requires C, 63·4; H, 6·05; N, 6·7%).

7-[2-(*Bis*-2-*chloroethylamino*)*ethyl*]-7H-*benzo*[c]*phenothiazine* (Vd).—Phosphorus oxychloride (21 ml.) was added slowly to the above amino-compound (7·05 g.) at 0°, after which the temperature of the mixture was allowed to increase gradually. The resulting dark solution was heated on a steam-bath for 75 min. The excess of phosphorus oxychloride was removed under reduced pressure, the residue dissolved in acetone, and the solution poured over excess of ice, neutralized with aqueous sodium carbonate, and extracted several times with benzene. The extracts were washed with water, concentrated, and chromatographed on a column of Florisil (3·0 × 45 cm.), with benzene as eluant, to yield the product as a viscous yellow oil (4·43 g., 57%).

The *hydrochloride* (73%) had m. p. 174·5—176·5° (Found: C, 58·2; H, 5·2; N, 6·15. $C_{22}H_{23}Cl_3N_2S$ requires C, 58·2; H, 5·1; N, 6·2%).

7-(2-*Chloroethyl*)-7H-*dibenzo*[c,h]*phenothiazine* (VIIIb).—7*H*-Dibenzo[*c*,*h*]phenothiazine was prepared according to the procedure of Knoevenagel [5] except that a maximum temperature of 165° was used. The yield was ~80% in several runs, and the product melted at 228—230° (decomp.). In an evacuated capillary it melted at 245—246° without decomposition.

The dibenzophenothiazine reacted with n-butyl-lithium and 2-chloroethyl toluene-*p*-sulphonate essentially in accordance with the procedure described above. The *product* (25%) melted at 184—185° (Found: C, 73·1; H, 4·65; N, 4·0. $C_{22}H_{16}ClNS$ requires C, 73·0; H, 4·5; N, 3·9%).

In the chromatographic separation of the above product, there was obtained by elution with a large volume of benzene a red crystalline material, m. p. 240—240·5°. Elemental analyses indicated that it was probably 1,2-*bis*-(7H-*dibenzo*[c,h]*phenothiazin*-7-*yl*)*ethane*, which would arise from the interaction of 7*H*-dibenzo[*c*,*h*]phenothiazin-7-yl-lithium and 7-(2-chloroethyl)-7*H*-dibenzo[*c*,*h*]phenothiazine (Found: C, 80·4; H, 4·5; N, 4·3. $C_{42}H_{28}N_2S_2$ requires C, 80·75; H, 4·5; N, 4·5%).

7-[2-(*Bis*-2-*hydroxyethylamino*)*ethyl*]-7H-*dibenzo*[c,h]*phenothiazine* (VIIIc).—Reaction of diethanolamine and 7-(2-chloroethyl)-7*H*-dibenzo[*c*,*h*]phenothiazine as described for compound (Vc) gave the *product* as yellow crystals (84%), m. p. 159—160° (Found: C, 72·6; H, 6·0; N, 6·4. $C_{26}H_{26}N_2O_2S$ requires C, 72·5; H, 6·1; N, 6·5%).

The *hydrochloride*, m. p. 256—261°, was prepared in 95% yield (Found: C, 66·5; H, 5·8; N, 6·1. $C_{26}H_{27}ClN_2O_2S$ requires C, 66·9; H, 5·8; N, 6·0).

7-[2-(*Bis*-2-*chloroethylamino*)*ethyl*]-7H-*dibenzo*[c,h]*phenothiazine* (VIIId).—Reaction of the hydroxy-compound with phosphorus oxychloride as described above gave bright yellow *needles* (67%), m. p. 117—118° (Found: C, 66·85; H, 5·1; N, 5·9. $C_{26}H_{24}Cl_2N_2S$ requires C, 66·8; H, 5·2; N, 6·0%).

The *hydrochloride* (88%), melted at 196—197·5° (*in vacuo*) (Found: C, 62·1; H, 5·0; N, 5·6. $C_{26}H_{25}Cl_3N_2S$ requires C, 62·0; H, 5·0; N, 5·6%).

12*H*-Benzo[*a*]phenothiazine and 14*H*-dibenzo[*a*,*h*]phenothiazine were similarly converted into the nitrogen-mustard derivatives (see Table).

12-(2-*Chloroethyl*)-12H-*benzo*[a]*phenothiazine* 7,7-*Dioxide*.—12-(2-Chloroethyl)-12*H*-benzo[*a*]-phenothiazine (0·60 g.) was dissolved in glacial acetic acid (70 ml.), 30% hydrogen peroxide (3·0 ml.) added, and the solution stirred at room temperature for 20 hr. The clear solution was poured into water (100 ml.), and the resulting suspension was broken by adding a small amount of magnesium sulphate and cooling. A dark red solid (0·42 g.) precipitated, and this was

[5] E. Knoevenagel, *J. prakt. Chem.*, 1914, **89**, 1.

crystallized from acetone–light petroleum (b. p. 66—75°) (charcoal) to give pale pink crystals (0·12 g., 18%), m. p. 149—150° (Found: C, 63·0; H, 4·0; N, 3·9. $C_{18}H_{14}ClNO_2S$ requires C, 63·0; H, 4·1; N, 4·1%).

Derivatives of 12*H*-benzo[*a*]- and 14*H*-dibenzo[*a*,*h*]-phenthiazine.

Compound	M. p.	Yield (%)	Found (%) C	H	N	Formula	Required (%) C	H	N
(VIa) [a]	134—136°	71							
(VIb)	101—102	35	69·3	4·5	4·3	$C_{18}H_{14}ClNS$	69·5	4·55	4·55
(VIc)	66—68	26	69·8	6·4	7·1	$C_{22}H_{24}N_2O_2S$	69·45	6·4	7·4
(VIc) dipicrate	145—148	—	49·1	3·8	12·8	$C_{34}H_{30}N_8O_{16}S$	48·7	3·6	13·3
(VId) hydrochloride	151—153	—[b]	58·0	5·2	6·0	$C_{22}H_{23}ClN_2S$	58·2	5·1	6·2
(VIIa) [c]	194—197 [d]	50—55							
(VIIb)	146—148	39	73·1	4·5	3·9	$C_{22}H_{16}ClNS$	73·0	4·4	3·9
(VIIc)	136—137 [e]	84	72·5	6·0	6·4	$C_{26}H_{26}N_2O_2S$	72·5	6·1	6·5
(VIIc) hydrochloride	211·5—213 [d]	49	66·7	5·9	6·0	$C_{26}H_{27}ClN_2O_2S$	66·9	5·8	6·0
(VIId)	108—109	54	66·75	5·2	5·7	$C_{26}H_{24}Cl_2N_2S$	66·8	5·2	6·0
(VIId) hydrochloride	175—185 [d]	88	61·9	5·0	5·7	$C_{26}H_{25}Cl_3N_2S$	61·9	5·0	5·6

[a] P. B. Talukdar and D. A. Shirley, *J. Amer. Chem. Soc.*, 1958, **80**, 3462. [b] Yield of the free base (an oil) was 25%. The hydrochloride was prepared from this in 85% yield. [c] F. Kehrman, A. Gressly, W. Chiffere, and M. Ramon, *Ber.*, 1923, **56**, 649. [d] *In vacuo*. [e] This compound apparently exhibited dimorphism, since a second form, m. p. 107—108°, was also isolated. Crystallization of the lower-melting form from ether–light petroleum gave the higher-melting form.

14-*Acetyl*-14H-*dibenzo*[a,h]*phenothiazine* (VII; R = Ac).—14*H*-Dibenzo[*a*,*h*]phenothiazine (5·0 g.) and anhydrous zinc chloride (3·0 g.) were added to acetic anhydride (40 ml.). The solution was stirred vigorously at room temperature for 15 min. and set aside for 24 hr., after which it was poured into a slurry of ice and water. The crude product crystallized from acetone–light petroleum (b. p. 60—90°) to yield white *crystals* (3·03 g., 53%), m. p. 191—193° (Found: C, 77·3; H, 4·3; N, 4·1. $C_{22}H_{15}NOS$ requires C, 77·4; H, 4·4; N, 4·1%).

14-*Acetyl*-14H-*dibenzo*[a,h]*phenothiazine* 7,7-*Dioxide*.—A solution of 14-acetyl-14*H*-dibenzo-[*a*,*h*]phenothiazine (1·0 g.) in glacial acetic acid (30 ml.) was treated with 30% hydrogen peroxide (2·0 ml.), gently warmed for 20 min., and heated under reflux for 90 min. The solution was cooled and pink crystals, (0·6 g., 55%), m. p. 240—241°, were removed by filtration. A portion of the product was redissolved in acetone and treated with activated charcoal, to yield white *crystals*, m. p. 246—248° (Found: C, 70·7; H, 4·1; N, 3·5. $C_{22}H_{15}NO_3S$ requires C, 70·8; H, 4·1; H, 3·8%).

14H-*Dibenzo*[a,h]*phenothiazine* 7,7-*Dioxide*.—10% Aqueous sodium hydroxide solution (0·5 ml.) was added to 14-acetyl-14*H*-dibenzo[*a*,*h*]phenothiazine 7,7-dioxide (0·5 g.) suspended in hot 95% ethanol (15 ml.). After a reflux period of 30 min., the solution was cooled, to yield a yellow solid (0·37 g., 83%), m. p. 407°. Recrystallization from acetone yielded cream-coloured *crystals*, m. p. 408°. The compound showed no apparent decomposition at the m. p. (Found: C, 71·7; H, 4·4. $C_{20}H_{13}NO_2S$ requires C, 72·5; H, 4·0%).

14-*Methyl*-14H-*dibenzo*[a,h]*phenothiazine* (VII; R = Me).—To a suspension of 14*H*-dibenzo-[*a*,*h*]phenothiazine (6·0 g.) in dry benzene (250 ml.) was added during 15 min. a pentane–heptane solution containing 0·02 mole of n-butyl-lithium. The resulting orange suspension of the *N*-lithio-salt was stirred for 1 hr., after which was added methyl toluene-*p*-sulphonate (5·6 g.) in benzene (20 ml.) The solution was refluxed for 20 hr., filtered, the solvent removed by evaporation, and the black viscous oil dissolved in benzene–light petroleum (6 : 4). The solution was chromatographed on Alcoa activated alumina (F-20 grade) using the same solvent as the eluant. Bright yellow *crystals* (3·8 g., 61%), m. p. 149—150°, were obtained from the first fraction (Found: C, 80·4; H, 4·7; N, 4·4. $C_{21}H_{15}NS$ requires C, 80·5; H, 4·8; N, 4·5%).

14H-*Dibenzo*[a,c]*phenothiazine* (IX).—9-Anilinophenanthrene was prepared from the reaction of potassium anilide and 9-bromophenanthrene in excess of aniline as the solvent. The mixture was heated under reflux for 15 hr., and distillation and two recrystallizations from light petroleum (b. p. 66—75°) yielded colourless needles (19·0 g., 40%), m. p. 133—134·5° (lit.,[6] 138°).

A mixture of 9-anilinophenanthrene (10 g.), sulphur (2·4 g.), and iodine (0·1 g.) was placed in an oil-bath at 155°. The first evolution of hydrogen sulphide was noted after the melt had

[6] A. Wolfram and W. Schnurr, G.P. 650,432/1937.

reached 142°. The mixture was then heated to 180—190°, and maintained at this temperature for 20 min., and then at 195—205° for 40 min. The warm melt was dissolved in benzene, and light petroleum (b. p. 100—115°) was added until a small amount of dark tar came out of solution. The resulting amber solution was chromatographed on Florisil; a large volume of light petroleum (b. p. 66—75°) eluted yellow needles (1·80 g., 16%), m. p. 160—164°. Two recrystallizations from light petroleum gave the product, m. p. 163—165° (Found: C, 79·9; H, 4·3; N, 4·6. Calc. for $C_{20}H_{13}NS$: C, 80·2; H, 4·4; N, 4·7%). This compound is reported in the literature;[7] however, no record of its properties or method of synthesis is available.

14-*Acetyl*-14H-*dibenzo*[a,c]*phenothiazine*.—To a mixture of acetic anhydride (2·0 ml.) and zinc chloride (0·20 g.) was added 14*H*-dibenzo[*a*,*c*]phenothiazine (0·20 g.). After 2 hr. at room temperature, the mixture was poured into ice–water. The precipitated brown solid was removed and crystallized from ethanol (charcoal) to yield the colourless *product* (0·15 g., 65%), m. p. 239—241°. Recrystallization from ethanol raised the m. p. to 242—243·5° (Found: C, 77·2; H, 4·2; N, 4·4. $C_{22}H_{15}NOS$ requires C, 77·4; H, 4·4; N, 4·1%).

14-*Methyl*-14H-*dibenzo*[a,c]*phenothiazine*.—14*H*-Dibenzo[*a*,*c*]phenothiazine (0·50 g.) was treated with n-butyl-lithium and methyl toluene-*p*-sulphonate as described for the dibenzo-[*a*,*h*]-analogue. The residual oil was taken up in light petroleum (b. p. 66—75°) and the solution chromatographed on Florisil. Light petroleum eluted the product slowly, and benzene–light petroleum (b. p. 100—115°) (3 : 7) proved to be a better eluant. The 14-*methyl*-14H-*dibenzo*-[a,c]*phenothiazine* (0·33 g., 63%), m. p. 118—119°, crystallized from light petroleum (b. p. 100—115°) after removal of the benzene (Found: C, 80·7; H, 5·0; N, 4·7. $C_{21}H_{15}NS$ requires C, 80·5; H, 4·8; N, 4·5%).

14-*Methyl*-14H-*dibenzo*[a,c]*phenothiazine* 9-*Oxide*.—A solution of 14-methyl-14*H*-dibenzo-[*a*,*c*]phenothiazine (0·11 g.) in glacial acetic acid (15 ml.) was treated with 30% hydrogen peroxide (1·50 ml.). The solution was warmed on a steam-bath for 10 min. and poured into water (100 ml.). The precipitate was recrystallized from acetone–light petroleum (b. p. 100—115°) to give the *product* (0·082 g., 71%), m. p. 206—207° (Found: C, 76·7; H, 4·6; N, 4·2. $C_{21}H_{15}NOS$ requires C, 76·6; H, 4·6; N, 4·25%). The infrared spectrum of the compound revealed bands at 9·63 and 9·81 μ, characteristic of the sulphoxide group.

14-(2-*Chloroethyl*)-14H-*dibenzo*[a,c]*phenothiazine* (IXb).—14*H*-Dibenzo[*a*,*c*]phenothiazine (0·42 g.) was treated with n-butyl-lithium and 2-chloroethyl toluene-*p*-sulphonate as described for the benzo[*c*]-analogue. The residual oil was dissolved in benzene–light petroleum (b. p. 100—115°) (2 : 3) and the solution was chromatographed on Florisil. Light petroleum (b. p. 100—115°) eluted most of the product and benzene–light petroleum (3 : 7) the remainder (total 0·26 g., 52%), m. p. 122·5—123° (from light petroleum) (Found: C, 72·7; H, 4·3; N, 3·7. $C_{22}H_{16}ClNS$ requires C, 73·0; H, 4·5; N, 3·9%).

Tests for carcinostatic activity of the compounds reported in this Paper are being obtained by the National Cancer Chemotherapy Service Center, Bethesda, Maryland; significant results will be reported elsewhere.

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Department of Chemistry, University of Tennessee,
Knoxville, Tennessee, U.S.A. [*Received, December 30th*, 1963.]

[7] A. Pacault, *Ann. Chim.* (*France*), 1946, [12], **1**, 565.