Notes

Synthesis of Oncolytic Analogs of 1,2-Dimethyl-4,5-diaminobenzene

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Recently, Woolley and Stewart have described the cure of some spontaneous mammary cancers of mice with certain analogs of 1,2-dimethyl-4,5-diaminobenzene (4,5-dimethyl-*o*-phenylenediamine).[1] The theoretical basis for the use of such compounds has been explained in a series of papers during the past decade.[1-4] In the present paper the synthesis of these compounds and of some of their less potent relatives is described. The most active substances were 1,2-dichloro-4-benzenesulfonamido-5-nitrobenzene (DCBN) (4',5'-dichloro-2'-nitrobenzenesulfonanilide) and 1,2-dimethyl-4,5-bis(benzenesulfonamido)benzene (DMDB) [N,N'-(4,5-dimethyl-*o*-phenylene)-bisbenzenesulfonamide]. DCBN was increased in oncolyticpotency by admixture with 1,2-dimethyl-4-(*p*-carboxyphenylazo)-5-hydroxybenzene [4-(4,5-dimethyl-2-hydroxyphenylazo)benzoic acid], the synthesis of which has already been described.[5]

Experimental

1,2-Dichloro-4-benzenesulfonamidobenzene (3',4'-Dichlorobenzenesulfonanilide), to Illustrate the General Method of Benzenesulfonylation.—A solution of 16.3 g. (0.1 mole) of 3,4-dichloroaniline in 50 ml. of pyridine was stirred and maintained at 20° while 12.8 ml. (0.1 mole) of benzenesulfonyl chloride was slowly added. After the solution had stood at room temperature for 0.5 hr. it was mixed with 200 ml. of aqueous *N* sodium hydroxide (to hydrolyze any remaining sulfonyl chloride) and held at room temperature for an additional 0.5 hr. It was then diluted with 200 ml. of water and acidified with 125 ml. of concentrated hydrochloric acid. The precipitate which formed was filtered, washed thoroughly with water, and recrystallized by solution in 100 ml. of acetic acid and dilution with 100 ml. of water. Physical and analytical data are shown in Table I.

Except as subsequently described, other arylsulfonylations were carried out by this general method. Thus, 1,2-dimethyl-4,5-bis(benzenesulfonamido)benzene was obtained from 1 equiv. of 1,2-dimethyl-4,5-diaminobenzene and 2.2 equiv. of benzenesulfonyl chloride, and the substituted sulfonylated compounds (such as the tosyl ones) were made with the appropriate sulfonyl chloride. The properties of these new compounds are given in Tables I and II.

1,2-Dimethyl-4-benzenesulfonamido-5-aminobenzene (2'-Amino-4',5'-dimethylbenzenesulfonanilide.)—A solution of 14 g. of 1,2-dimethyl-4,5-diaminobenzene in 50 ml. of pyridine was cooled to 0° and slowly treated with 13 ml. of benzenesulfonyl chloride with stirring. The reaction mixture was allowed to warm to 20° for 1 hr. and was then treated with 250 ml. of aqueous *N* sodium hydroxide. After 30 min. the pH was adjusted to 5 with hydrochloric acid and 250 ml. of water was added. When the precipitate had solidified it was collected, washed thoroughly with water, and suspended in 150 ml. of ethanol. The suspension was refluxed for 15 min., cooled to 25°, and filtered immediately. The insoluble portion (the diacylated compound, m.p. 218°, 3.8 g.) was discarded, and the desired product was precipitated from the alcohol by evaporation to 50 ml. and addition of 100 ml. of water. It was then recrystallized from ethanol–water. Physical and analytical data are given in Table II.

1,2-Dichloro-4-benzenesulfonamido-5-nitrobenzene (4',5'-Dichloro-2'-nitrobenzenesulfonanilide.)—Powdered 1,2-dichloro-4-benzenesulfonamidobenzene (60 g.) was slowly added with stirring to 400 ml. of yellow fuming nitric acid (sp. gr. 1.48) which had been precooled to −15°. The addition was slow enough that the temperature did not rise above −5°. One-half hour after the last addition, the clear solution was poured into 5 kg. of ice–water. The precipitate which formed was filtered, washed thoroughly with water, dried, and recrystallized to constant melting point from glacial acetic acid (300 ml.).

The position of the nitro group in this compound was proved by reduction (as described later) to the amine, followed by benzenesulfonylation to yield the known 1,2-dichloro-4,5-bis(benzenesulfonamido)benzene [N,N'-(4,5-dichloro-*o*-phenylene)bisbenzenesulfonamide)], m.p. and m.m.p., 183–185°.

1,2-Dichloro-4-benzenesulfonamido-5-aminobenzene (2'-Amino-4',5'-dichlorobenzenesulfonanilide).—To a stirred solution of 68 g. of 1,2-dichloro-4-benzenesulfonamido-5-nitrobenzene in 300 ml. of boiling acetic acid was added 850 ml. of concentrated hydrochloric acid and 50 g. of mossy tin. The tin was added slowly so that the reaction did not become too vigorous. The mixture was stirred and heated until all the tin had dissolved. The solution was then concentrated under reduced pressure to 350 ml. Addition of 1 l. of water completed precipitation of the desired amine. The amine was such a weak base that its hydrochloride did not form in the aqueous acid. The free base was filtered, washed with water, and recrystallized from 50% ethanol. The starting material was almost insoluble in this solvent so that purification was easily effected.

1,2-Dimethyl-4-bromo-5-succinamidobenzene [N-(2-Bromo-4,5-dimethylphenyl)succinamic acid].—A solution of 0.20 g. of 6-bromo-3,4-xylidine and 0.14 g. of succinic anhydride in 5 ml. of dry pyridine was allowed to stand at room temperature for 3 days and was then concentrated to low volume, and the residue was taken up in dilute sodium hydroxide solution. After a small amount of the starting material (the amine) had been removed by filtration, the product was precipitated by acidification of the filtrate with hydrochloric acid, and was purified by one recrystallization from benzene.

1,2-Dichloro-4-benzenesulfonamido-5-succinamidobenzene [N-(2-Benzenesulfonamido-4,5-dichlorophenyl)succinamic Acid].—An intimate mixture of 6.3 g. of 1,2-dichloro-4-benzenesulfonamido-5-aminobenzene and 2.2 g. of succinic anhydride was stirred and heated in an oil bath at 150° for 1 hr. The mixture first melted and then crystallized. The cooled magma was triturated at 100° with 200 ml. of water and 4 g. of sodium bicarbonate. Unchanged starting material (800 mg.) was removed by filtration. The product was obtained by acidification of the filtrate to pH 1, collection of the precipitate, and recrystallization from ethanol–water.

Other succinyl and phthalyl derivatives, listed in Tables I and II, were prepared by the same general procedure as described for the foregoing compound by use of the proper amine and anhydride.

1,2-Dimethyl-4-bromo-5-succinoxybenzene (2-Bromo-4,5-dimethylphenyl Hydrogen Succinate).—A solution of 0.5 g. of 6-bromo-3,4-xylenol, 0.5 g. of succinic anhydride, and 3 drops of concentrated sulfuric acid in 10 ml. of dry dioxane was allowed to stand for 3 days at room temperature and was then concentrated to 5 ml. The residue was diluted with 30 ml. of 5% sodium carbonate solution, and the resulting solution was chilled, acidified with hydrochloric acid, and extracted with ether. The product obtained after drying and evaporation of the ether was an oil which crystallized when scratched. The crystals were triturated with petroleum ether (b.p. 30–60°), filtered, and washed well with petroleum ether. Recrystallization from methylcyclo-

(1) D. W. Woolley and J. M. Stewart, *Biochem. Pharmacol.*, **11**, 1163 (1962). "Cure" means that the spontaneous cancers vanished and did not reappear throughout the period of observation, which was 5 months from the time of disappearance of the tumors. All spontaneous cancers were not cured, but a considerable percentage were.

(2) D. W. Woolley, *Proc. Natl. Acad. Sci. U. S.*, **39**, 6 (1953).

(3) D. W. Woolley, *ibid.*, **41**, 111 (1955).

(4) D. W. Woolley, *Cancer Res.*, **13**, 327 (1953).

(5) D. W. Woolley, *J. Am. Chem. Soc.*, **74**, 5450 (1952).

TABLE I

DERIVATIVES OF 1,2-DICHLOROBENZENE

Abbreviation	Substituents	Yield, %	M.p., °C.	% Calcd. C	% Calcd. H	% Calcd. N	% Found C	% Found H	% Found N
	4-Benzenesulfonamido-	80	125–126	47.6	3.0	4.6	48.0	3.2	4.5
DCBN	4-Benzenesulfonamido-5-nitro-	52	131–134			8.1			8.0
DCBA	4-Benzenesulfonamido-5-amino-	74	158	45.5	3.1	8.8	45.5	3.1	8.9
DCBS	4-Benzenesulfonamido-5-succinamido	72	158–160	46.0	3.4	6.8	45.9	3.5	6.8
DCBT	4-Benzenesulfonamido-5-phthalimido-	92	169–170	51.3	3.0	6.0	51.3	2.6	6.2
DCDB	4,5-Bisbenzenesulfonamido	85	183–185	47.2	3.0		47.5	3.2	
	4-Benzenesulfonamido-5-*p*-toluenesulfonamido-	71	175	48.5	3.4	6.0	48.2	3.6	5.7
	4-Acetamido-5-hydroxy-[a]	45	212–214	43.7	3.2		43.8	3.2	

[a] Prepared from 2-amino-4,5-dichlorophenol[6] by acetylation.

TABLE II

DERIVATIVES OF 1,2-DIMETHYLBENZENE

Abbreviation	Substituents	Yield, %	M.p., °C.	% Calcd. C	% Calcd. H	% Calcd. N	% Found C	% Found H	% Found N
DMBA	4-Benzenesulfonamido-5-amino-	72	148–150	61.0	5.5	10.4	61.0	5.6	10.2
DMDB	4,5-Bisbenzenesulfonamido-	66	218–220	57.6	4.8	6.7	57.9	4.8	6.8
DMBS	4-Benzenesulfonamido-5-succinamido-	77	160–161	57.3	5.4	7.5	57.2	5.3	7.6
	4-Bromo-5-succinamido-	70	170–171.5	48.0	4.7	4.7	48.2	4.8	4.8
	4-Bromo-5-succinoxy-	48	103–104	47.8	4.4		48.3	4.4	
	4,5-Bisbenzylamino-	81	88–90	83.5	7.6	8.9	83.6	7.6	9.0

hexane gave a pure product. Unchanged starting material (the xylenol, 0.2 g.) was recovered from the petroleum ether.

1,2-Dimethyl-4,5-bis(benzylamino)benzene (N,N′-Dibenzyl-4,5-dimethyl-*o*-phenylenediamine).—N,N′-(4,5-Dimethyl-*o*-phenylene)bisbenzamide[7] (3.44 g.) was stirred with a suspension of 1.50 g. of lithium aluminum hydride in 500 ml. of dry ether at room temperature for 6 days. Excess hydride was decomposed by cautious addition of aqueous potassium sodium tartrate, and the ether layer was separated, dried, and evaporated. The residue was recrystallized from ether–petroleum ether (b.p. 30–60°).

2-Bromo-4,5-dimethylphenoxyacetic acid was prepared from 1.0 g. of 6-bromo-3,4-xylenol[8] by the standard method for converting phenols to aryloxyacetic acids.[9] Recrystallization of the product from ethanol–water gave 0.4 g. of prisms, m.p. 142–142.5°. Much of the starting material (0.6 g.) was recovered unchanged.

Anal. Calcd. for $C_{10}H_{11}BrO_3$: C, 46.3; H, 4.2. Found: C, 46.5; H, 4.5.

1-Ribityl-2-benzyl-5,6-dimethylbenzimidazole.—A mixture of 3.06 g. of 4,5-dimethyl-*N*-ribityl-*o*-phenylenediamine hydrochloride,[10] 1.01 g. of triethylamine, and 2.00 g. of ethyl phenylacetimidate hydrochloride (prepared from phenylacetonitrile) in 25 ml. of dry dioxane was stirred at 35° for 18 hr. The solvent was evaporated and the residue was dissolved in dilute hydrochloric acid. The solution was chilled and made basic with ammonium hydroxide, and the precipitated product was filtered, washed well with water, dried, and recrystallized from ethanol; yield 3.0 g. (81%), m.p. 196–198°.

Anal. Calcd. for $C_{21}H_{26}N_2O_4$: C, 68.1; H, 7.1; N, 7.3. Found: C, 67.9; H, 7.1; N, 7.4.

5,6-Dimethyl-2-(4-nitrobenzyl)benzimidazole was prepared by the general method used for the preceding compound from 6.9 g. of 4,5-dimethyl-*o*-phenylenediamine, 12.3 g. of ethyl *p*-nitrophenylacetimidate hydrochloride, and 5.1 g. of triethylamine in 75 ml. of dioxane; yield 5.5 g. (40%) of yellow prisms, m.p. 208–210°.

Anal. Calcd. for $C_{16}H_{15}N_3O_2$: C, 68.3; H, 5.4. Found: C, 68.3; H, 5.3.

1-Benzyl-5,6-dimethylbenzimidazole.—5,6-Dimethylbenzimidazole[7] (1.46 g.) was converted to the sodium salt in dry ethanol with 0.24 g. of sodium hydride. Benzyl chloride (1.26 g.) was added, the solution refluxed for 2 hr., and then cooled. After the sodium chloride had been filtered off, the filtrate was concentrated and chilled to yield 1.0 g. of the desired product, m.p. 190–192°.

Anal. Calcd. for $C_{16}H_{16}N_2$: C, 81.3; H, 6.8. Found: C, 81.1; H, 6.9.

2,3-Dihydroxy-6,7-dimethylquinoxaline.—A mixture of 5 g. of 4,5-dimethyl-*o*-phenylenediamine and 50 ml. of diethyl oxalate was refluxed for 2 hr. The cooled suspension was centrifuged and the precipitate was washed thoroughly with ethanol. It was then extracted several times with hot aqueous *N* sodium hydroxide, and the desired compound was precipitated from the alkaline extract by acidification. The yield was 6.5 g., unmelted at 350°.

Anal. Calcd. for $C_{10}H_{10}N_2O_2$: C, 63.2; H, 5.3. Found: C, 62.9; H, 5.2.

6,7-Dimethyl-3-hydroxy-2-quinoxalinylcarbonylurea.—A mixture of 13.6 g. of 4,5-dimethyl-o-phenylenediamine and 16.0 g. of alloxan monohydrate in 100 ml. of water was heated, with stirring, until no further precipitation occurred. The product was filtered off, washed well with ethanol, and dried yielding 24.0 g. of yellow crystals, m.p. 267° dec. These crystals were stirred with hot dimethylformamide, filtered, washed with dimethylformamide and ether, and dried, m.p. 274° dec. The structure of this compound was deduced from the work of King and Clark-Lewis,[11] who made some related compounds.

Anal. Calcd. for $C_{12}H_{12}N_4O_3$: C, 55.4; H, 4.7; N, 21.5. Found: C, 55.3; H, 5.7; N, 21.4.

6,7-Dimethyl-2-hydroxyquinoxaline.—6,7-Dimethyl-3-hydroxy-2-quinoxalinylcarbonylurea (2.6 g.) was warmed in *N* sodium hydroxide solution until evolution of ammonia was complete. The solution was chilled and acidified, and the precipitated 6,7-dimethyl-3-hydroxy-2-quinoxalinecarboxylic acid

(6) D. W. Woolley and A. Pringle, *J. Biol. Chem.*, **194**, 729 (1952).
(7) N. G. Brink and K. Folkers, *J. Am. Chem. Soc.*, **71**, 2951 (1949).
(8) A. W. Crossley and D. J. Bartlett, *J. Chem. Soc.*, **103**, 1297 (1913).
(9) R. L. Shriner and R. C. Fuson, "The Systematic Identification of Organic Compounds," 3rd Ed., John Wiley and Sons, Inc., New York, N. Y. 1948, p. 210.
(10) Generously supplied by Hoffmann-La Roche, Inc.
(11) F. E. King and J. W. Clark-Lewis, *J. Chem. Soc.*, 3379 (1951).

was filtered, washed well with water, and dried; wt. 2.2 g., m.p. 284–295° dec. A solution of 1.5 g. of this carboxylic acid in 25 ml. of redistilled quinoline was refluxed for 1 hr., cooled, and poured into ice–dilute hydrochloric acid. The precipitate was collected by filtration, washed well with water, and dried; wt., 1.15 g., m.p. 289–292°. Recrystallization from glacial acetic acid gave pure product, m.p. 292–293°.

Anal. Calcd. for $C_{10}H_{10}N_2O$: C, 69.0; H, 5.8; N, 16.1. Found: C, 68.7; H, 6.1; N, 16.2.

Biological Activity Against Spontaneous Mammary Cancers.—The ability of some of the foregoing compounds to cause regression and cure of spontaneous mammary cancers of SPFS mice has already been described.[1] The most active found was a mixture of 1,2-dichloro-4-benzenesulfonamido-5-nitrobenzene and 1,2-dimethyl-4-*p*-carboxyphenylazo-5-hydroxybenzene.[5] Similar assay of most of the other compounds in the series has shown that they were either less active or were without activity against these spontaneous cancers.

Substituted 2-Aminobenzimidazoles[1]

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As part of our continuing studies on the physical and physiological properties of benzimidazole derivatives[2] we wish to report at this time a number of hitherto undescribed 2-aminobenzimidazoles and a preliminary report of their physiological activity. Although several methods for the synthesis of 2-aminobenzimidazoles have been reported,[3] remarkably few of these have been described.[4] This is somewhat surprising in view of the simplicity of synthesis and the fact that this type of compound is of chemical interest considered as disubstituted guanidines.

After screening for circulatory effects of the compounds the most marked effect was observed on the conductive mechanism of the heart. Administered intravenously to cats and rabbits it was found that 2-aminobenzimidazole and the methyl-substituted derivatives caused a defect in the normal conductive processes of the heart as indicated by a 50–100% increase in the QRS interval and a 30–50% increase in the PR interval of the electrocardiogram.[5] 2-Amino-5-chlorobenzimidazole and 2-amino-1-phenylbenzimidazole were ineffective under the same experimental conditions. Increased activity was noted among the effective compounds with increased substitution of methyl groups in the benzene ring. A methyl group in the 1-position generally decreased effectiveness.

Experimental

For the synthesis of the present compounds we have utilized the procedure in which an appropriate *o*-phenylenediamine is condensed with cyanogen bromide.[3b] We have found (in distinction to the previous workers) that the reaction mixture can be worked up after 15 min. and that ammonium hydroxide may be used instead of sodium hydroxide to isolate the product from the acidic reaction mixture. Otherwise the procedure is the same as the one previously reported. The compounds prepared in this work are shown in Table I. The first five compounds listed were synthesized from *o*-phenylenediamines obtained commercially. The others were synthesized from known N-methyl-*o*-phenylenediamines prepared from appropriate *o*-nitroaniline by the series of reactions: tosylation,[6] methylation,[6] detosylation,[6] and reduction.[7]

Biological Testing.—Male and female cats and rabbits weighing 2–3 kg. were anesthetized lightly with sodium pentothal. The right common carotid artery and the external jugular vein were isolated and the two vessels cannulated proximally and tied off. The cannula in the jugular vein was attached to a Phipps-Bird constant injection apparatus and the cannula in the carotid artery was connected to a Sanborn pressure transducer. Electrodes were placed on the animal's legs and the electrocardiogram and blood pressure recorded on a Sanborn Twin-Viso recorder. The compounds were tested by dissolving them in distilled water with the aid of hydrochloric acid (pH of the final solution, 5–6). The animals served as their own controls. When the blood pressure became stabilized and the animal registered a normal electrocardiogram the test compound was administered at a rate of 5 mg./min./kg. in 0.2 ml. of solution.

TABLE I

SUBSTITUTED 2-AMINOBENZIMIDAZOLES

2-Aminobenzimidazole	Average yield, %	M.p.,[a] °C.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found[b]
5-(or 6-)CH_3	65	203–204[c]	$C_8H_9N_3$	65.31	65.20	6.12	6.12
5,6-$(CH_3)_2$	60	228–229	$C_9H_{11}N_3$	67.08	67.37	6.83	6.83
4,6-(or 5,7)-$(CH_3)_2$	55	215–216	$C_9H_{11}N_3$	67.08	67.22	6.83	6.79
5-(or 6)-Cl	60	169–170[d]					
1-C_6H_5	60	154–155	$C_{13}H_{11}N_3$	74.64	74.36	5.26	5.35
1-CH_3	75	202–203[e]	$C_8H_9N_3$	65.31	65.58	6.12	6.47
1,5-$(CH_3)_2$	57	235–236	$C_9H_{11}N_3$	67.08	66.83	6.83	6.80
1,5,6-$(CH_3)_3$	83	259–260	$C_{10}H_{13}N_3$	68.57	68.80	7.43	7.66

[a] Fisher–Johns hot stage. [b] Analyses by C. F. Geiger, Ontario, Calif. [c] Lit.[3e] m.p. 196–197. [d] Lit.[3b] m.p. 167–168°. [e] Lit.[3e] m.p. 200–201.

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(1) This investigation was supported by the San Diego County Heart Association and in part by Public Health Service Research Grant HS 6792.

(2) L. Joseph and J. Julca, *J. Org. Chem.*, **27**, 1101 (1962).

(3) (a) P. Pierron, *Ann. Chim.*, [8] **15**, 191 (1908); (b) N. J. Leonard, D. Y. Curtin, and K. M. Beck, *J. Am. Chem. Soc.*, **69**, 2459 (1947); (c) L. S. Efros, B. A. Porai-Koshits, and S. G. Farbenstein, *J. Gen. Chem. USSR*, **23**, 1961 (1953); (d) N. D. Vitkevich and A. M. Simonov, *ibid.*, **29**, 2578 (1959); (e) N. P. Bednyagina and I. Ya. Postovskii, *ibid.*, **30**, 1456 (1960).

(4) A considerable number of benzimidazole carbamic acids and their esters have been reported in the patent literature, *e.g.*, H. L. Klopping, U. S. Patent 2,933,504 (1960), and H. M. Loux, U. S. Patent 3,010,968 (1961). These compounds may be looked upon as 2-aminobenzimidazoles which have been substituted on the amino group. The present compounds contain an intact amino group.

(5) G. E. Burch and T. Winsor, "A Primer of Electrocardiography," 4th Ed., Lea and Febiger, Philadelphia, Pa., 1960, p. 86.

(6) E. H. Usherwood and M. A. Whitely, *J. Chem. Soc.*, 1084 (1923).

(7) D. Balcom and A. Furst, *J. Am. Chem. Soc.*, **75**, 5334 (1953).