Acta Chemica Scandinavica B 32 (1978) 317—321

Hydrophilic Derivatives of Benzidine. A Novel Group of Chromogens with Reduced Handling Risks

N. Å. JÖNSSON, F. MERENYI, C. M. SVAHN and J. GYLLANDER

Department of Organic Chemistry, Research Department, AB Kabi, S-112 87 Stockholm, Sweden

A series of ω,ω'-(4,4'-diamino-3,3'-biphenyl)- and ω,ω'-(4,4'-diamino-3,3'-biphenylylenedioxy)-dialkanoic and dialkanesulfonic acids is prepared as potentially less carcinogenic substitutes for benzidine and benzidine derivatives in analytical application.

Benzidine and some of its derivatives, notably *o*-tolidine and *o*-dianisidine, have already long ago proven their usefulness as analytical reagents. These compounds, especially the first two, are, however, also potent carcinogens in animals, and have reportedly caused bladder cancer even in laboratory workers exposed to small amounts of the substances.[1] Increasing concern with the health hazards produced by chemical carcinogens has resulted in drastic regulations for the handling of benzidine.[2] *o*-Tolidine and *o*-dianisidine are not yet subject to the same restrictions but it has been repeatedly urged that extreme care be exercised in any handling of them and that safer substitutes should be used whenever possible.[3–5] A number of non-benzidine type compounds has also been suggested as alternatives, but it has, however, been difficult to find substitutes for the benzidines without sacrificing at least partially the favourable properties inherent in these substances. One tempting approach to effective and less hazardous reagents appeared to be "molecular manipulation" in order to reduce or hopefully obliterate the dangerous properties of the established benzidines instead of searching for non-benzidine type compounds, and it is the purpose of this paper to describe such attempts. One similar study has recently been published.[6]

In our opinion, any successful substitute for the conventional benzidine chromogens must fulfil the following requirements:

1. Its performance in analytical applications must be equally good or better than that of the parent compound.

2. Its physical properties should reduce the risk of unintentional uptake by inhalation or by resorption through the skin (a major portal of entry for benzidine and its lipophilic analogues [7]) and should facilitate removal by ordinary washing procedures and speed up excretion of the substance without prior metabolic conversion.

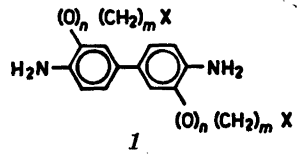
3. Its toxicity and carcinogenicity must be so low that exceptional, accidental exposure to reasonable amounts of the compound does not constitute any unacceptable risk.

The requirements formulated in paragraph 2 suggests that molecular manipulations should be tried that convert the lipid-soluble, rather volatile parent compounds into high-melting, highly ionized water-soluble derivatives. Such transformations would definitely reduce the risk of inhalation and skin penetration and could also be expected to facilitate excretion and reduce metabolic transformations. As it is generally believed that metabolites, notably hydroxylamine derivatives, rather than the parent amines themselves, are the immediate carcinogenic agents,[8] facilitated elimination and/or decreased metabolic transformation could also be expected to reduce the carcinogenic potential of the compounds if these are nevertheless resorbed. From these considerations it follows that the introduction of substituents such as carboxyl or sulfonic acid groups into

the molecules should be tried, a principle already successfully used to convert highly carcinogenic azo dyes to comparatively nontoxic dyestuffs.

The utility of the benzidines as chromogens depends on their conversion upon oxidation to highly coloured products. The ease of this reaction and the position and intensities of the absorption bands of the coloured products formed vary considerably between the derivatives as a function of their substitution pattern. It can therefore be concluded that the electron distribution of the parent compounds has to be retained principally intact, which strongly limits the possibilities for molecular manipulation. The introduction of polar groups directly into the benzene rings can thus *a priori* be excluded, as it would fundamentally change the redox potential of the compounds, a conclusion that was born out by experiments with benzidine-3,3′-dicarboxylic acid and 3,3′-dimethoxybenzidine-6,6′-disulfonic acid and suggests the methyl groups of *o*-tolidine and *o*-dianisidine as sites for manipulations.

As a result of these considerations the general structure *1* emerged

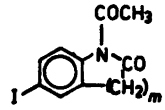


1

1a	$n=1$ $m=3$	X = COOH	(Method: A, C)
1b	$n=1$ $m=3$	X = SO_3H	(Method: A)
1c	$n=1$ $m=4$	X = SO_3H	(Method: A)
1d	$n=0$ $m=3$	X = COOH	(Method: B, C)
1e	$n=0$ $m=4$	X = COOH	(Method: B)

where $n=0$ or 1, m an integer and X a sulfonic or carboxylic acid group. In order to minimize the influence on the rings of the strongly polar X group, m should be at least 2. The well-known spontaneous formation of 5- and 6-membered lactams, which would reduce the stability of the carboxylic acid derivatives in near neutral solutions, introduced the additional condition that for these acids n+m should be not less than 3.

Three different procedures were used for the syntheses: A, Alkylation of 4,4′-bisacetamido-3,3′-dihydroxybiphenyl with an appropriate halocarboxylic acid or sultone derivative, when necessary followed by hydrolysis. B, Ullman coupling of an *N*-acetyliodolactam, *2*, $m=3$ or 4, to the corresponding biphenyl derivative followed by hydrolytic opening of the lactam rings. C, Reduction of an appropriate *o*-nitrophenylalkyl or *o*-nitrophenoxyalkyl carboxylic or sulfonic acid to the corresponding hydrazobenzene derivative followed by benzidine rearrangement. The compounds prepared and the methods used are listed under structure *1*.



2

The choice of method for the individual compounds was mainly based on the availability of suitable starting materials.

Method A is very versatile but involves the handling of supposedly carcinogenic 3,3′-dihydroxybenzidine and its *N*,*N*′-diacetyl derivative and in some cases carcinogenic sultones.

This disadvantage is avoided in method C which, however, may afford mixtures of isomeric derivatives, which may be difficult to separate.

It is noteworthy that the Ullman reaction could not be carried out with the iodolactams unsubstituted at the nitrogen atom but required protection by *N*-acetylation and then afforded about 60 – 65 % yields.

The compounds were screened for utility as analytical reagents and *1a*, γ,γ′-(4,4′-diamino-3,3′-biphenylylenedioxy)dibutyric acid (suggested trivial name *dicarboxidine*), was selected as a superior chromogen for a number of analytical applications. The properties of this compound and the results of extensive testing of its carcinogenic potential will be reported separately.[9]

EXPERIMENTAL

Melting points were taken on a Leitz microscope hot plate apparatus. All compounds were routinely checked by IR and ^1H NMR spectroscopy and results were in agreement with those theoretically expected. Microanalyses were carried out by AB Analytica, Solna, Sweden.

Method A

γ,γ'-(4,4'-Diamino-3,3'-biphenylylenedioxy)dibutyric acid. 1a. Dimethyl γ,γ'-(4,4'-diacetamido-3,3'-biphenylylenedioxy)dibutyrate. To a suspension of sodium hydride (16 g; 0.60 mol) in anhydrous dimethylformamide (800 ml) 4,4'-diacetamido-3,3'-dihydroxybiphenyl[10] (90 g; 0.30 mol) was added in small portions with stirring under nitrogen at about −5 °C. After completed addition the mixture was stirred until the evolution of hydrogen had stopped (about 1 h), whereupon methyl γ-chlorobutyrate (90 g; 0.66 mol) was added dropwise at about 0 °C. The temperature was slowly increased to 80 °C and maintained overnight. Most of the solvent was distilled off under reduced pressure and the residue poured into 2 l of ice water. The crude ester was collected and crystallized from 2 l of 2-propanol affording 110 g (73 %) of crude product. A sample was further recrystallized and then melted at 159–162 °C. Anal. $C_{28}H_{32}N_2O_8$: C, H, N.

γ,γ'-(4,4'-Diacetamido-3,3'-biphenylylenedioxy)dibutyric acid. The crude ester (74 g; 0.15 mol) was added portionwise at 20 °C under nitrogen to a stirred solution of sodium hydroxide (27 g; 0.66 mol) in water (450 ml) and methanol (400 ml). The solution was kept for 20 h at room temperature, diluted with water (5 l), filtered and acidified with 2 N hydrochloric acid. The product was filtered off and crystallized from dilute acetic acid. Yield 73 %. M.p. 214–217 °C. Anal. $C_{24}H_{28}N_2O_8$: C, H, N.

γ,γ'-(4,4'-Diamino-3,3'-biphenylylenedioxy)dibutyric acid dihydrochloride. The acetamido compound (40 g; 0.085 mol) was refluxed for 6 h with 2 N hydrochloric acid (1 l). The hot solution was treated with carbon and filtered. On cooling a solid precipitated, which was collected and recrystallized from 2 N hydrochloric acid. Yield 31 g (80 %) of slightly greyish crystals which decomposed at 165 °C. Anal. $C_{20}H_{26}Cl_2N_2O_6$: C, H, Cl, N.

The free *amino acid* was obtained when the dihydrochloride (0.92 g) dissolved in water (10 ml) was treated with pyridine (0.32 g). The product was filtered off, washed with water and dried at room temperature. Microcrystalline powder containing hydrate water, which was given off at about 65 °C. The anhydrous product decomposed without definite m.p. at about 160 °C. Anal. $C_{20}H_{24}N_2O_6$: C, H, N.

3,3'(-4,4'-Diamino-3,3'-biphenylylenedioxy)dipropanesulfonic acid, 1b. 4,4'-Diacetamido-3,3'-dihydroxybiphenyl (90.1 g; 0.30 mol) was added to sodium hydroxide (26.7 g; 0.66 mol) dissolved in methanol (500 ml) followed by a suspension of 1,3-propanesultone (73.3 g; 0.60 mol) in methanol (200 ml). The mixture was stirred and heated for 1 h at 85 °C and then cooled to about 5 °C. The precipitated disodium 3,3'-(4,4'-diacetamido-3,3'-biphenylylenedioxy)dipropanesulfonate (108 g; 61 %) was filtered off and dried.

The dry product (33 g; 0.056 mol) was dissolved in water (100 ml) and passed through a cation exchange column (Amberlite IR-120; H^+-saturated). The filtrate was concentrated to about 50 ml, added to concentrated hydrochloric acid (450 ml) and refluxed for 2 h. On cooling 3,3'-(4,4'-diamino-3,3'-biphenylylenedioxy)dipropanesulfonic acid precipitated and was collected by filtration. A second crop was obtained on concentration of the mother liquor, giving a total yield of 16.5 g (64 %). An analytically pure product was obtained when this crude product was crystallized from water. It decomposed at about 300 °C. Anal. $C_{18}H_{24}N_2O_8S_2$: C, H, N.

4,4'-(4,4'-Diamino-3,3'-biphenylylenedioxy)dibutanesulfonic acid, 1c was similarly prepared from 1,4-butanesultone. Microcrystalline powder which decomposed at about 270 °C. Anal. $C_{20}H_{28}N_2O_8S_2$: C, H, N, S.

Method B

γ,γ'-(4,4'-Diamino-3,3'-biphenyl)dibutyric acid, 1d. 7-Iodo-2-oxo-2,3,4,5-tetrahydro-1H-1-benzazepine. Iodine monochloride (32.5 g; 0.2 mol) in acetic acid (200 ml) was added with stirring to a solution of 2-oxo-2,3,4,5-tetrahydro-1*H*-1-benzazepine[11] (32.2 g; 0.2 mol) in acetic acid (300 ml). The mixture was kept at room temperature for 48 h and diluted with water (about 2 l). The precipitate was collected and crystallized from ethanol. Yield 26.8 g; 47 %. M.p. 188–189 °C. Anal. $C_{10}H_{10}INO$: C, H, N.

N-Acetyl-7-iodo-2-oxo-2,3,4,5-tetrahydro-1H-1-benzazepine. The iodo lactam (10 g; 0.035 mol) was refluxed for 6 h with acetic anhydride (160 ml), then evaporated under reduced pressure to afford a thick oil which crystallized on trituration with methanol. The product melted at 116–117 °C. Yield 9.5 g (83 %). Anal. $C_{12}H_{12}INO_2$: C, H, I, N.

7,7'-Bis-(2-oxo-2,3,4,5-tetrahydro-1H-1-benzazepine). The *N*-acetyl lactam (9.4 g; 0.028 mol) was agitated with copper powder (20 g) in anhydrous dimethylformamide (100 ml) under reflux. After 3 h an additional 20 g of copper powder was added and heating and agitation was continued for 12 h. Solid material was filtered off and washed with hot dimethylformamide. The combined filtrate and washings were concentrated *in vacuo* to a small volume. Addition of water precipitated a solid product which was dissolved in 50 % aqueous methanol, made slightly alkaline with 2 N sodium hydroxide solution and filtered from some precipitated contaminant. Acidification with 2 N hydrochloric acid caused crystallization of pure product. Yield 3.0 g (65 %). The product melts at 178 °C, resolidifies and decomposes at about 300 °C. Anal. $C_{20}H_{20}N_2O_6$: C, H, N.

γ,γ'-(4,4'-Diamino-3,3'-biphenyl)dibutyric acid. The bis-benzazepine derivative (1 g) was heated for 7 h at 150 °C in an autoclave with

150 ml of aqueous barium hydroxide solution saturated at room temperature. After cooling, sodium carbonate solution was added to precipitate barium carbonate which was filtered off and carefully washed with water. Acidification of the filtrate with acetic acid afforded the amino acid as colourless crystals which melt at about 120 °C with loss of crystal water. Anal. $C_{20}H_{24}N_2O_4 \cdot H_2O$: C, H, N.

δ,δ'-(4-4'-Diamino-3,3'-biphenyl)divaleric acid, 1e. This compound was prepared from 2-oxo-1,2,3,4,5,6-hexahydro-1-benzazocine [12] as described above for the dibutyric acid analogue.

8-Iodo-2-oxo-1,2,3,4,5,6-hexahydro-1-benzazocine melts at 207 – 208 °C. Anal. $C_{11}H_{12}INO$: C, H, I, N.

The subsequent steps were carried out without isolation of the intermediates in pure state. The bis-benzazocine derivative was hydrolyzed with refluxing concentrated hydrochloric acid and the amino acid was precipitated from the acid solution with saturated aqueous sodium carbonate solution at pH 6. The product contained 1 mol of crystal water which was given off at about 150 °C. Anal. $C_{22}H_{28}N_2O_4 \cdot H_2O$: C, H, N.

Method C

γ,γ'-(4,4'-Diamino-3,3'-biphenyl)dibuturic acid, 1d. γ-(2-Nitrophenyl)butyric acid. Diethyl malonate (50 g; 0.27 mol) was added to sodium hydride (6.5 g; 0.27 mol) in dimethylformamide (60 ml) cooled with ice-water. When the evolution of hydrogen ceased, 2-(*o*-nitrophenyl)-ethyl chloride (37.1 g; 0.20 mol) was added and the mixture was heated at 60 °C with stirring for 2 h. After cooling, the mixture was acidified with 2 N hydrochloric acid, the oil that separated was extracted into chloroform, washed with water and dried. Evaporation of the solvent and some unreacted diethyl malonate *in vacuo* left an oil (54.5 g) consisting of a mixture of mono- and dialkylated malonic esters. It was dissolved in a mixture of methanol (1250 ml), water (100 ml) and potassium hydroxide (45 g) and left overnight at room temperature. A solid precipitate (mainly dialkylated ester) was filtered off. The filtrate was concentrated to dryness on a water bath under reduced pressure, the solid residue was dissolved in water, washed with ether and acidified with hydrochloric acid. The acid solution was repeatedly extracted with ether, the combined extracts were dried (Na_2SO_4) and the solvent evaporated affording a semicrystalline residue (24.3 g) which on treatment with chloroform afforded the pure *[2-(o-nitrophenyl)ethyl]malonic acid*, m.p. 138 °C. This acid (3.8 g; 0.015 mol) was refluxed for 2 1/2 h with 65 % sulfuric acid. Dilution with water and cooling afforded a crystalline product which was collected and crystallized from 50 % aqueous acetic acid. Yield 2.5 g (72 %) of γ-(2-nitrophenyl)butyric acid, m.p. 70 °C. Anal. $C_{10}H_{11}NO_4$: C, H, N.

The compound has previously been isolated as a by-product from the nitration of γ-phenylbutyric acid and reported to melt at 55 – 57 °C.[13]

γ,γ-(4,4'-Diamino-3,3'-biphenyl)dibutyric acid. Zinc powder (15 g) was added in small portions during 5 min to a solution of γ-(2-nitrophenyl)butyric acid (4.2 g; 0.02 mol) in 6 N sodium hydroxide solution (25 ml). The temperature rose quickly and was kept at 90 °C for an additional 10 min. Solid zinc salts were removed by filtration under nitrogen (sintered glass filter) and the filtrate immediately added to 6 N hydrochloric acid (100 ml). Some solid material that was immediately formed was removed by filtration. The filtrate was diluted with 6 N hydrochloric acid causing the diamino acid *dihydrochloride* to precipitate. This was filtered off after cooling the mixture to about 5 °C. Reprecipitation from its water solution with concentrated hydrochloric acid yielded 1.5 g (35 %) of almost colourless crystals. Anal $C_{20}H_{26}Cl_2N_2O_4$: C, H, Cl, N.

γ,γ'-(4,4'-Diamino-3,3'-biphenylylenedioxy)dibutyric acid, 1a. γ-(2-Nitrophenoxy)butyric acid. Ethyl γ-chlorobutyrate (33.0 g; 0.22 mol) was added to a mixture of *o*-nitrophenol (27.8 g; 0.20 mol), anhydrous potassium carbonate (30.3 g; 0.22 mol) and dimethylformamide (100 ml) and stirred for 20 h in a boiling water bath. Undissolved salts were removed by filtration and washed with dimethylformamide. The combined filtrate and washings were concentrated to about 75 ml under reduced pressure and diluted with water (150 ml). The ester which precipitated as an oil was separated, washed with water, diluted with ethanol (500 ml) and added in portions to stirred 10 % aqueous sodium hydroxide solution (200 ml). Stirring was continued until a clear solution was obtained (about 1 h) which was then strongly acidified with concentrated hydrochloric acid. The crystalline product was collected, washed with water and dried. Yield 52.5 g (94 %). M.p. 103 °C. It may be crystallized from toluene. Anal. $C_{10}H_{11}NO_5$: C, H, N.

γ,γ'-(4,4'-Diamino-3,3'-biphenylylenedioxy)dibutyric acid. The dihydrochloride was obtained when γ-(2-nitrophenoxy)butyric acid was reduced and rearranged as described above for compound *1d*. Yield 40 % of almost colourless crystals, identical by m.p., IR and ^1H NMR spectroscopy with the product prepared by method A.

REFERENCES

1. Searle, C. E. *Chem. Ber. 6* (1970) 5.
2. Department of Labor, Occupational Safety and Health Administration: *Carcinogens, Occupational Health and Safety Standards, Fed. Regist. 39* (1974) 3755.
3. The Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer

Hospital: *Precautions for laboratory workers who handle carcinogenic aromatic amines*, London 1966.
4. George, W. H. S. *Ann. Clin. Biochem. 8* (1970) 130.
5. Cluley, H. J. *Analyst 99* (1974) 81.
6. Holland, V. R. Saunders, B. C., Rose, F. L. and Walpole, A. L. *Tetrahedron 30* (1974) 3299.
7. Meigs, J. W., Sciarini, L. J. and van Sondt, W. A. *Arch. Ind. Hyg. Occup. Med. 9* (1954) 122.
8. Uehleke, H. *Arzneim.-Forsch. 19* (1969) 1033.
9. Jönsson, N. Å., Gröningsson, K., Pavlu, B., Vessman J. and Westlund, L.-E. *In preparation.*
10. Burkhardt, G. N. and Wood, H. *J. Chem. Soc.* (1929) 141.
11. Huisgen, R. and Ugi, I. *Justus Liebigs Ann. Chem. 610* (1957) 57, p. 65.
12. Hjelte, N. and Agback, T. *Acta Chem. Scand. 18* (1964) 191.
13. Reppe, W. *Justus Liebigs Ann. Chem. 596* (1955) 219.

Received February 10, 1978.