Antiulcer Effect of the *N*- and *O*-β-D-Glucopyranosides of 5-Aminosalicylic Acid

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Key Words: *5-Aminosalicylic acid; Mesalazinum; O- and N-glucosides; Crohn disease; colitis ulcerosa*

Summary

Starting from methyl 5-nitrosalicylate (**20**) the *N*- and *O*-β-glucopyranosyl derivatives (**24, 28**) of 5-aminosalicylic acid were prepared. The LD_{50} values of these compounds were determined on mice, and the inhibitory effect of **24** (0.83 mmol/kg) and **28** (1.2 mmol/kg) on gastric ulcer on rats, induced by indomethacin was investigated.

Introduction

5-Aminosalicylic acid (**2**, 5-ASA) was first synthesized at the end of the last century, and was employed first of all for the production of azo dyes. Recently, 5-ASA was identified [1–5] as the structural unit of numerous antifungal, antibacterial, and antitumor antibiotics. Of the sulfonamide preparations *Salazopyrin*® (**1**), soluble in the small intestines, is built up from 5-ASA (**2**) and sulfapyridine (**3**). Earlier studies [6,7] have shown that in the large intestine **1** is decomposed into **2** and **3** upon the action of the azoreductase enzymes of colic bacteria, and these compounds are further metabolized to **4** and **5**, respectively (Figure 1).

It has also been demonstrated [8,9] that the effective component in treatment of intestinal inflammatory diseases (such as *Crohn disease*, *colitis ulcerosa*) is not sulfapyridine (**3**), but it is the 5-ASA (**2**) portion of the molecule. This recognition contributed to the application of 5-ASA as a non-steroidal anti-inflammatory agent in therapy in various medical preparations, such as *Salofalk*® and *Pentasa*,® etc., which are marketed and administered in different formulations (tablet, suppository).

To improve the pharmacokinetic and pharmacodynamic effect of 5-ASA, several prodrug preparations (Figure 2) are known in the literature. By analogy with *Salazopyrin*, 4-(3′-carboxy-4′-hydroxyphenylazo)benzenesulfonamidopyridine (**6**) was synthesized, and also reported [10] to possess an immunosuppressive effect. In compound **7**, 5-ASA is linked

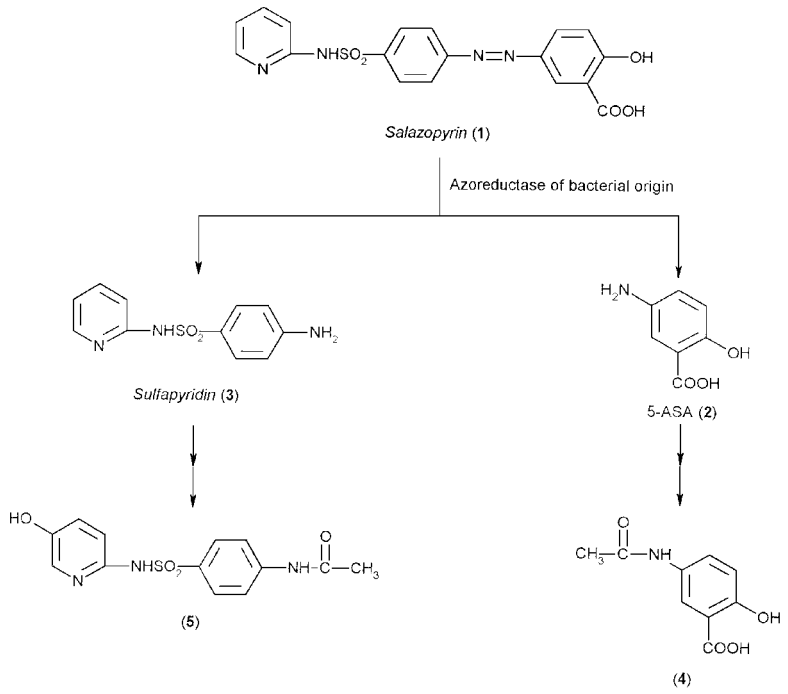

Figure 1. *In vivo* metabolism of Salazopyrin.

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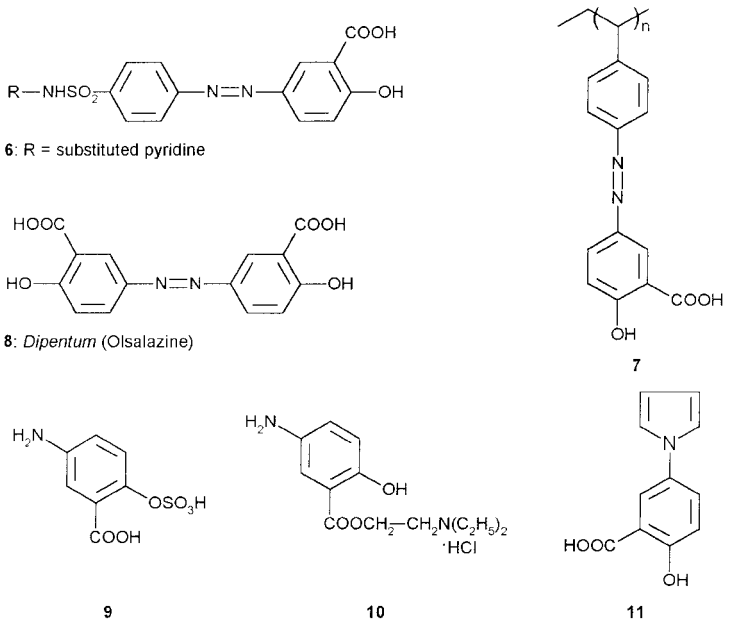

Figure 2. Pro-drug derivatives of 5-ASA.

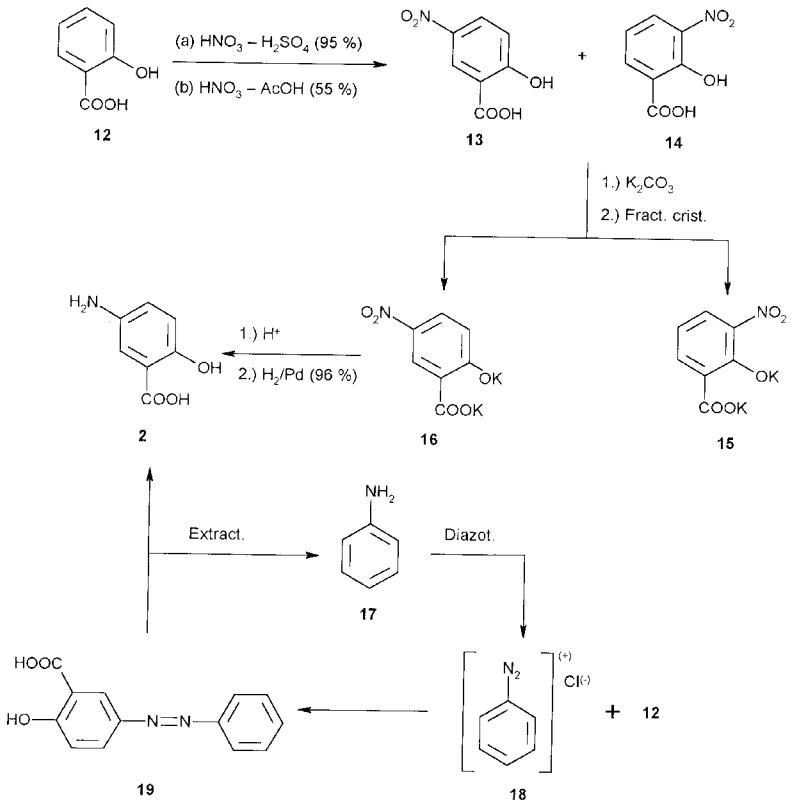

Figure 3. Preparation of 5-aminosalicylic acid (**2**).

to a polystyrene skeleton [11], and the molecule of *Dipentum*® [**8**, 3,3-azobis-6-hydroxybenzoic acid (Olsalazin)] is built up [12] from two 5-ASA units.

The effective material (5-ASA) is produced from all three compounds **6–8** upon the action of the azoreductase enzyme of colic bacteria. The most advantageous derivative is **8**, since reductive decomposition of the molecule produces 5-ASA exclusively. In a German Patent[13] *O*-sulfonyl-5-ASA (**9**) is proposed for the treatment of *Colitis ulcerosa*, but others [14] prefer administration of the novocaine derivative **10**. 5-*N*-Pyrrolylsalicylic acid was expected [15] to be a potential antipyretic, analgetic and anti-inflammatory agent. Several *N*-acyl derivatives of 5-ASA, possessing various pharmacological effects, have also been prepared [16].

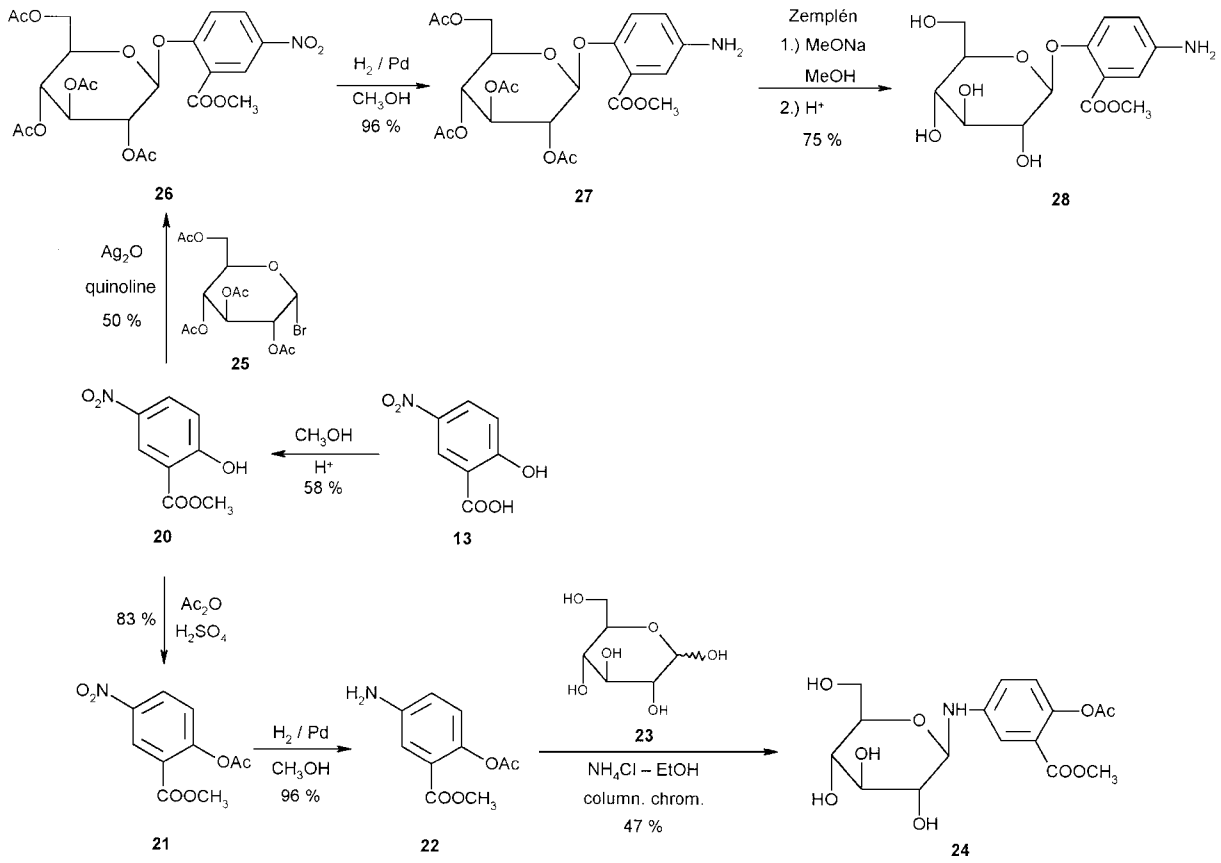

Figure 4. Synthesis of the *N*- and *O*-β-D-glucopyranoside derivatives of 5-aminosalicylic acid (**24** and **28**).

Although the mechanism of action of 5-ASA (**2**) is not known in all details, it is clear that – similarly to indomethacin and acetylsalicylic acid – it inhibits the cyclooxygenase [17] enzyme involved in the biosynthesis of prostaglandins, as well as that of the soybean lipoxygenase [18] enzyme. On the other hand, they inhibit cellular transcription factors that are involved in the inducible expression of a variety of cellular genes that regulate the inflammatory response [37]. In the present paper we report on the synthesis (Figure 4) and results of the preliminary biological investigation of the stable and water-soluble *N*-and *O*-β-D-glucoside derivatives (**24, 28**) of 5-ASA, which are expected to possess prodrug properties.

Results and Discussion

Preparation of 5-Aminosalicylic Acid

According to a long-known procedure [19], salicylic acid (**12**) is nitrated with a mixture of concentrated sulfuric acid and nitric acid to furnish a 1:1 mixture of 5-nitro (**13**) and 3-nitrosalicylic acid (**14**) (Figure 3). However, when the same reaction of **12** is carried out [20] in glacial acetic acid containing 10% of concentrated nitric acid, a 3:1 mixture of **13** and **14** is produced. The difference between the water solubility [21] of the bis-potassium salts **15** and **16,** derived from the acids, allows the separation of the isomers: upon cooling a hot aqueous solution of the two compounds **15** is precipitated and **16** remains in solution. Following acidification, the separated 5-nitrosalicylic acid is converted into **2** by means of catalytic hydrogenation or reduction with sodium dithionite. An efficient and economic synthesis[22] of 5-ASA involves diazotization of aniline (**17**) and reaction of the resulting phenyldiazonium chloride (**18**) with salicylic acid to furnish 5-phenylazosalicylic acid (**19**). Reduction of this latter compound then gives the desired 5-ASA (**2**), and aniline (**17**) which can be recycled into the process.

For the reduction of **19** various procedures have been reported [22–24], and our patented method [25] involves catalytic hydrogenation over palladium-on-carbon in aqueous suspension, or in methanolic or acetic acid solution. The product **2** forms intensely coloured complexes with heavy metals, which is very much undesired concerning the appearance and utility of the product in medical preparations. Therefore, the crude product was occasionally purified [26] by dissolving in an acid solution (pH 60 2) in the presence of complex-forming materials, such as ethylenediamine tetraacetic acid, etc., and subsequent precipitation with ammonium hydroxide at pH = 3–3.5. In connection with our synthetic work, an HPLC method [27] was elaborated for the detection and quantitative determination of 5-ASA in drug preparations and in human biological fluids.

Synthesis of the Glucosides of 5-Aminosalicylic acid

Treatment of **13** with abs. methanol in the presence of sulfuric acid under reflux gave the methyl ester **20**, which was acetylated with acetic anhydride to yield methyl 2-acetoxy-5-nitrobenzoate (**21**). Catalytic hydrogenation of **21** over palladium-on-carbon catalyst led to methyl 2-acetoxy-5-aminobenzoate (**22**). Then glucosylation of **22** (Fig. 4) was accomplished with D-glucose in refluxing dry ethanol in the presence of ammonium chloride,[28] and the resulting methyl 2-acetoxy-5-(β-D-glucopyranosylamino) benzoate (**24**) was purified on a Kieselgel 60 column with an 8:2 benzene-methanol eluent.

In the ^{1}H NMR spectrum of **24** the $J_{1,2}$ = 8.5 Hz coupling constant indicated the presence of the β-D-glucopyranosyl moiety. Removal of the acetoxy and methyl ester units at the aromatic ring of **24** was regarded unnecessary, as these are *in vitro* readily hydrolysed by the human esterase enzymes.

Preparation of the 2-*O*-β-glucoside of 5-ASA was also carried out using compound **20**. Thus, reaction of **20** with α-acetobromo-D-glucose (**25**) was performed in a heterogeneous phase, in the presence of silver oxide and quinoline promoter[29] at room temperature to give **26**. The nitro group of **26** was converted into amino by catalytic hydrogenation in methanol, and Zemplén *O*-deacetylation of the produced **27** furnished methyl 2-*O*-β-D-glucopyranosyl-5-amino benzoate (**28**). The β-configuration of the anomeric hydroxyl group in **28** was unequivocally proved by the ^{1}H NMR ($J_{1,2}$=8 Hz) and ^{13}C NMR spectral data ($^1J_{C\text{-}1,H\text{-}1}$=159.4 Hz).

Pharmacology

The extremely low solubility (1 mg/ml), and instability of 5-ASA[19–21, 25] is quite disadvantageous with respect to the medical utilization and resorption. In contrast, the glycosides **24** and **28** are stable compounds, with water solubility ten times and one hundred times, respectively, higher than that of the parent compound **2**.

The LD_{50} values (Table 1) of the *N*- and *O*-β-D-glucopyranoside derivatives (**24** and **28**) were determined on mice by using 5-ASA as the reference compound. Upon intraperitoneal and oral administration, respectively, the glycosides **24** and **28** were found to be four times and one and a half times less toxic than 5-ASA.

The severity of the gastric ulcer induced by indomethacin was investigated on rats relative to a control group of animals (n = 14), and the results (Table 2) are expressed with the ulcer index values (for definition see the Experimental Part). The data of Table 2 shows the ulcer inhibition effect (inhibition % relative to 5-ASA) found for **24** and **28**. The glycoside **24** possesses a dose-dependent inhibitory effect. Administration of 0.83 mmol/kg of **24** resulted in an ulcer inhibition effect practically identical with that observed with 1.63 mmol/kg of 5-ASA (**2**). Thus, the *N*-glycoside **24** is effective in a half-dose of 5-ASA.

Contrary to the above findings, a 1.2 mmol/kg dose of **28** caused a much weaker inhibitory effect, which is most certainly a consequence of the general biotransformation pathway of salicylic acid derivatives. It is believed that further experiments are needed to determine if **24** is suitable for the treatment of ulcerogenic damages on the basis of its more soluble and less toxic character than the referential substance 5-ASA [33–35].

Table 1. LD_{50} values of 5-ASA (**2**) and its *N*- and *O*-β-D-glucopyranoside derivatives (**24** and **28**) on mice.

	Administration (mg/kg)	
Compound	i.p.	p.o.
5-ASA (**2**)	469 (348–630)	3370 (2884–3939)
24	> 2000	> 5000
28	> 2000	> 5000

Table 2. The inhibitory effect of 5-ASA (**2**) and its *N*- and *O*-β-D-glucopyranoside derivatives (**24** and **28**) on gastric ulcer on rats induced by indomethacin.

Compound	Number of rats (n) (mmol/kg)	i.p. Dose	% Decrease of ulcer index relative to control
5-ASA (**2**)*	5	1.63	–48.8
	10	0.27	–32.3
24	9	0.55	–38.6
	9	0.83	–46.3
28	5	1.20	–25.0

Ulcer index value of control: 28.5 ± 2.7.
* Applied in a suspension of 0.9% aqueous NaCl.

Acknowledgement

The present work was financially supported by the BIOGAL Pharmaceutical Works (Debrecen, Hungary), and by the Grant OTKA T 029075 given by the National Fund for Scientific Research Development (Budapest, Hungary).

Experimental Part

Melting points were determined on a Kofler hot-stage apparatus and in capillary tubes and are uncorrected. Specific optical rotations were measured with a Perkin-Elmer 241 automatic polarimeter. ^{1}H- and ^{13}C NMR spectra were recorded with a Bruker WP 200 SY instrument at 200 and 50.3 MHz, respectively (internal Me_4Si). Thin layer chromatography was carried out on Kieselgel 60 F_{254} (DC-Alurolle, Merck) precoated layers, and column chromatography was performed on Kieselgel 60 (Merck, 0.2–0.5 mm) with the following solvent mixtures: (A) 1:1 benzene-ethyl acetate; (B) 7:3:3:3 ethyl acetate-(*n*-butanol)-acetic acid-water; (C) 8:2 benzene-methanol. The thin layer chromatograms were visualized with ethanol containing 5% of sulfuric acid (120 °C), 5% ferric(III) chloride solution, or with a 0.2% solution of ninhydrin in ethanol.

*Methyl 5-nitrosalicylate (**20**)*

5-Nitrosalicylic acid (**13,** 5.49 g, 30 mmol), prepared according to literature procedures [20, 21], was dissolved in abs. methanol (60 ml) by gentle heating, and to this solution concentrated sulfuric acid (1.2 ml) was dropwise added. The reaction mixture was boiled under reflux for 9 h, cooled to room temperature, the precipitated crystals were filtered off (4.04 g) and recrystallized from methanol to obtain 3.42 g of pure **20** (57.86 %). Mp = 118–119 °C, R_f = 0.80 (solvent mixture A). Anal. $C_8H_7NO_5$ C, H, N.

*Methyl 2-Acetoxy-5-nitrobenzoate (**21**)*

To a solution of **20** (1.79 g, 0.01 mol) in acetic anhydride (20 ml) four drops of concentrated sulfuric acid were added, and the reaction mixture was heated on a steam bath with exclusion of humidity for 3 h. The brown solution was then poured onto crushed ice (400 g), the brown precipitate was filtered off and air-dried. The crude product (2.30 g) was crystallized from ether-petroleum ether to obtain pure **21** (1.99 g, 83.2%), mp = 73–74 °C, R_f = 0.86 (solvent mixture A). Anal. $C_{10}H_9NO_6$ C, H, N.

*Methyl 2-Acetoxy-5-aminobenzoate (**22**)*

A mixture of 10% palladium-on-carbon catalyst (0.53 g) in abs. methanol (20 ml) was saturated with hydrogen gas, a solution of **21** (3.41 g, 0.014 mol) in abs. methanol (100 ml) was added, and the mixture was hydrogenated under atmospheric pressure for 3.5 h. The catalyst was then filtered off, the filtrate was concentrated under diminished pressure, and the residue was crystallized from hot water (60 ml) with decolorization with charcoal. The product was dried (IR lamp) to obtain 2.04 g (67.54%) of pure **22**, mp = 103–105 °C, R_f = 0.57 (solvent mixture A). Anal. $C_{10}H_{11}NO_4$ C, H, N.

*Methyl 2-Acetoxy-5-(β-D-glucopyranosylamino)benzoate (**24**)*

To a solution of **22** (1.04 g, 5 mmol) in abs. methanol (150 ml) pre-dried **23** (0.90 g, 5 mmol) and ammonium chloride (0.08 g, 1.5 mmol) were added, and the mixture was boiled under reflux for 10 h. It was then decolorized with charcoal, filtered, concentrated under reduced pressure, and the residue was purified by column chromatography (solvent system E). The combined eluate was evaporated, and the residue was crystallized from a mixture of chloroform, ether, and petroleum ether to isolate **24** as pale yellow plates, yield: 0.84 g (46.9%). Mp = 153–155 °C, $[\alpha]_D^{23}$= –84.4° (*c* = 0.64, H_2O), R_f = 0.18 (solvent mixture C). Anal. $C_{15}H_{21}NO_9$ C, H, N. ^{1}H NMR (DMSO-d_6-D_2O) δ: 7.23 (1H, m, H_c), 6.62 (1H, d, NH), 5.0 (1H, d, OH), 4.90 (2H, t, OH), 4.46 (1H, t, OH), 4.36 (1H, t, H-1, $J_{1,2}$=8.5 Hz), 3.75 (3H, s, $COOCH_3$), 3.62–3.10 (6H, m, H-2, H-3, H-4, H-5, H-6a, H-6b), 2.20 (3H, s, OC-CH_3).

*Methyl 2-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosyl-oxy)-5-nitrobenzoate (**26**)*

To a homogenized mixture of **20** (3.94 g, 0.02 mol), **25** (16.44 g, 0.04 mol) and activated silver oxide (9.25 g, 0.04 mol) quinoline (15 ml) was added, the mixture was vigorously stirred together, whereupon it solidified in an exothermic reaction. After 1 h, the mass was homogenized with glacial acetic acid (100 ml), and the brown suspension was poured onto crushed ice. The precipitate was filtered off, washed with ice-water, dried and crystallized from ethanol (170 ml) to yield 5.24 g (49.7%) of pure **26** as yellow crystals. Mp = 163–165 °C, $[\alpha]_D^{23}$ = –36.8° (*c* = 0.8, $CHCl_3$), R_f = 0.70 (solvent mixture A). Anal. $C_{22}H_{25}NO_{15}$ C, H, N. ^{1}H NMR ($CDCl_3$) δ: 8.68 (1H, d, H_c), 8.35 (1H, dd, H_b), 7.25 (1H, d, H_a), 5.50–5.13 (4H, m, H-1, H-2, H-3, H-4), 4.25 (2H, m, H-6a, H-6b), 4.03 (1H, m, H-5), 3.95 (3H, s, OCH_3), 2.07 (12H, 4s, CO-CH_3).

*Methyl 2-(β-D-glucopyranosyl-oxy)-5-aminobenzoate (**28**)*

A mixture of 10% palladium-on-carbon catalyst (0.18 g) in ethyl acetate (10 ml) was saturated with hydrogen gas, a solution of **26** (1.2 g, 2.28 mmol) in ethyl acetate (40 ml) was added, and hydrogenation was continued at atmospheric pressure for 3 h. The mixture was then filtered, concentrated under diminished pressure, and the syrupy residue was crystallized form ethyl acetate-petroleum ether to yield pure **27** (1.09 g, 96.46%), mp = 138 °C, $[\alpha]_D^{23}$ = –25.2° (*c* = 0.5, $CHCl_3$), R_f = 0.32 (solvent mixture A). Anal. $C_{22}H_{27}NO_{12}$ C, H, N.

The product **27** (2.90 g, 5.83 mmol) was dissolved in abs. methanol (150 ml) and 15 drops of a 1 M sodium methoxide solution in methanol was added. After 1 h, the mixture was adjusted to ca. pH 6, decolorized with charcoal, and concentrated to ca. 15 ml under reduced pressure. Storage of the solution at +4 °C resulted in crystallization of **28**; it was filtered off and dried under reduced pressure. Yield: 1.44 g (75%), mp = 156 °C (in a capillary tube), $[\alpha]_D^{23}$ = –43.7° (*c* = 0.54, H_2O), R_f = 0.47 (solvent mixture B). Anal. $C_{14}H_{19}NO_8$ C, H, N. ^{1}H NMR (DMSO-d_6): δ: 7.05 (1H, d, *ortho*-aromatic proton, $J_{1,2}$ = 8 Hz), 6.9 (1H, d, *meta*-aromatic proton, $J_{1,2}$ = 2 Hz), 6.7 (1H, dd, *meta*-aromatic proton), 4.9–5.1 (5H, m, 4 OH and anomeric H-1), 4.2–4.46 (2H, m), 3.78 (3H, $COOCH_3$), 3.7 (1H, m), 3.5 (1H, m), 3.1–3.3 (4H, m).

Acute Toxicity

The approximate acute toxicity of 5-ASA (**2**), and its glucosylated derivatives **24** and **28** was determined on a group of 10 CFLP mice/dose according to the method described by Turner.[30] The reference material was 5-ASA (**2**), purchased from Nobel Chemicals, and the LD_{50} values were calculated according to Litchfield and Wilcoxson.[36].

Induction of Gastric Ulcer

Male Sprague-Dawley rats of 180–250 g were housed at controlled room temperature and humidity (20–22 °C, 45–60% relative humidity), and fed with a standard diet of laboratory chow and tap water. The animals were fasted for 18 h before drug administration, but allowed free access to water.

The indomethacin-type ulcer method was employed[31,32]: 30 mg/kg *Indomethacin* (Sigma) prepared in 0.9% NaCl, was administrated orally to the animals. The ulcerogenic challenge was given 30 min after either saline administration (control group), or administration of the test substances prepared in 0.9% NaCl (treated groups), intraperitonally.

After 4 h indomethacin treatment, the animals were killed by ether anaesthesia. The stomach was removed and dissected along the greater curvature under an illuminated magnifier. The mucosal damage was macroscopically examined and expressed in terms of the ulcer index, which was calculated according to the method [32] of Rainsford: i.e. ulcer index = mean of lesion number + stomach lesion severity + ulcer incidence. The severity of lesions was scored from 0–4 on an artificial scale: 0 = no ulcer; 1 = superficial mucosal lesion; 2 = deep ulcer; 3 = long and deep ulcer; 4 = penetrating ulcer. Inhibition % was calculated on the basis of reduction in ulcer index between the control and treated groups.

References

[1] H. Takai, M. Yoshida, T. Iida, I. Matsubara, K. Shiramata, *J. Antib*. **1976**, *29*, 1253–1257.

[2] T. Sasaki, K. Furimata, H. Nakayama, H. Seto, N. Otake, *Tetrahedron Lett*. **1981**, *27*, 1603–1607

[3] H. H. Wasserman, R. J. Gambale, *J. Am. Chem. Soc*. **1985**, *107*, 1423–1424.

[4] M. Konishi, H. Ohkuma, K. Saitoh, H. Kawaguchi, *J. Antib*. **1985**, *38*, 1605–1609.

[5] M. Prudhomme, G. Dauphin, G. Jeminet, *J. Antib*. **1986**, *39*, 923–933.

[6] M. A. Peppercorn, P. Goldman, *J. Pharm. Exp. Ther*. **1972**, *181*, 595–598.

[7] A. K. A. Khan, G. Guthrie, H. H. Johnstone, S. C. Truelove, D. H. Williamson, *Clin. Sci*. **1983**, *64*, 349–354.

[8] A. K. A. Khan, J. Piris, S. C. Truelove, *Lancet*, **1977**, *2*, 892–895.

[9] S. N. Rasmussen, V. Binder, K. Maier, S. Bondesen, C. Fischer, V. Klotz, S. H. Hansen, E. F. Hridberg, *Gastroenterology*, **1983**, *85*, 1350–1353.

[10] K. H. Agback, R. E.Lindbolm, E. Rangvald, DBP. 2257629. **1971** (November 26).

[11] T. M. Parkinson, J. P. Brown, R. E. Wingard, USP. 4190716. **1980** (February 26).

[12] CH. 632.670. **1977**.

[13] H. Rokos, H. Konczak, W. Forth, DBP. 3323702. **1983** (January 7).

[14] E. Evans, DBP. 722795. **1950** (July 31).

[15] L. H. Sarett, W. W. Ruyle, GBP. 1359560. **1972** (February 25).

[16] E. Bouley, J. M. Teulon, M. Cazes, A. Cloarec, R. Deghenghi, *J. Med. Chem*. **1986**, *29*, 100–103.

[17] W. F. Stenson, E. Lobos, *Biochem. Pharmacol*. **1983**, *32*, 2205–2209.

[18] H. Allgayer, J. Eisenburg, G. Paumgartner, *Eur. J. Clin. Pharmacol*. **1984**, *26*, 449–451.

[19] R. Hirsch, *Chem. Ber*. **1900**, *33*, 3238–3241.

[20] H. C. Bárány, M. Pianka, *J. Chem. Soc*. **1946**, 965–966.

[21] R. G. Taborsky, USP. 3278372. **1965** (July 2).

[22] A. Fischer, G. F. Schaar-Rosenberg, *Chem. Ber*. **1899**, 32, 81–84.

[23] N. Puxeddu, *Gaz. Chim. Ital*. **1929**, *59*, 12–15.

[24] E. Grandmougin, *Chem. Ber*. **1906**, *39*, 3929–3932.

[25] I. Gáspár, G. Szabó, F. Sztaricskai, I. Csípõ, A. Kiss, S. Jancsó, I. Takács, K. Marossy, Hung.P. 22211/88. **1988** (October 17).

[26] I. Gáspár, G. Szabó, F. Sztaricskai, I. Csípõ, A. Kiss, S. Jancsó, I. Takács, K. Marossy, Hung.P. 22210/88. **1988** (October 17).

[27] E. Nagy, I. Csípõ, G. Szabó, I. Degrell, *J. Chromat*. **1988**, *425*, 214–219.

[28] R. Bognár, P. Nánási, É. P. Nánási, *Magy. Kém. Foly*. **1953**, *59*, 185–191.

[29] A. Robertson, R. B. Waters, *J. Chem. Soc*. **1930**, 2729–2733.

[30] R. A. Turner, *Screening Methods in Pharmacology*. Academic Press, New York, London, **1965**.

[31] P. DelSoldato, C. Foschi, L. Varin, S. Daniotti, *Agent Actions*, **1985**, *16*, 393–396.

[32] K. D. Rainsford, *Gut*. **1975**, *16*, 514–527.

[33] B. M. Peskar, K. W. Dreyling, B. May, K. Schaarschmidt, H. Goebll, *Digest. Dis. Sci*. **1987**, *32*, 516–565.

[34] U. Klotz, K. E. Maier, *Digest. Dis. Sci*. **1987**, *32*, 465–505.

[35] E. Ezer, L. Szporny, *J. Pharm. Pharmacol*. **1981**, *33*, 250–251.

[36] J. R. Litchfield, F. Wilcoxson, *J. Pharmacol. Exp. Ther*. **1949**, *96*, 99.

[37] M. J. Yin, Y. Yamamoto, R. B. Gaynor, *Nature* **1998**, *396*, 77–80.

Received: April 21, 1999 [FP388]