Aspirin Prodrugs: Synthesis of 2-Substituted 2-Methyl-4*H*-1,3-benzodioxin-4-ones and their Screening for Prodrug Potential

Karin Kramer Nielsen and Alexander Senning*

Department of Organic Chemistry, University of Aarhus, DK-8000 Århus C, Denmark

Nielsen, K. K. and Senning, A., 1990. Aspirin Prodrugs: Synthesis of 2-Substituted 2-Methyl-4*H*-1,3-benzodioxin-4-ones and their Screening for Prodrug Potential. – Acta Chem. Scand. 44: 952–956.

A series of new 2-substituted 2-methyl-4*H*-1,3-benzodioxin-4-ones **1** have been synthesized and fully characterized. This study involves fifteen compounds of which fourteen are orthoesters, containing tertiary aliphatic alkoxy groups. One compound contains a *tert*-butylperoxy group and one a 3β-cholesteryloxy group in the 2-position. The hydrolysis of these compounds **1** was followed in enzymatic and non-enzymatic media to clarify whether they are true prodrugs of aspirin. Two compounds **1** were additionally tested *in vivo* as potential topical keratolytics.

Aspirin[1] **2**, which, originally, was prepared as a prodrug[2] for salicylic acid **3**, is now one of the most widely used drugs. With pharmacodynamics related to its antiinflammatory, analgesic, antipyretic and antithrombotic properties[3] **2** is (although relatively high doses are required) a highly effective and inexpensive drug. Unfortunately, **2** has disadvantages that make its oral use, which is the most common way of administration, problematic for many patients, especially those with gastrointestinal ulcers or gastric sensitivity. These disadvantages of **2** are largely a local phenomenon, possibly involving accumulation of the acid inside the gastric mucosal cells.[3]

Our goal is to improve the therapeutic index by developing non-acidic prodrugs of aspirin. The 2-methyl-2-oxy-4*H*-1,3-benzodioxin-4-one structure **1** is a promising approach, because **2** is latent here. Whether **1** is a prodrug for aspirin or salicylic acid or both, depends on which bonds are broken first upon hydrolysis.

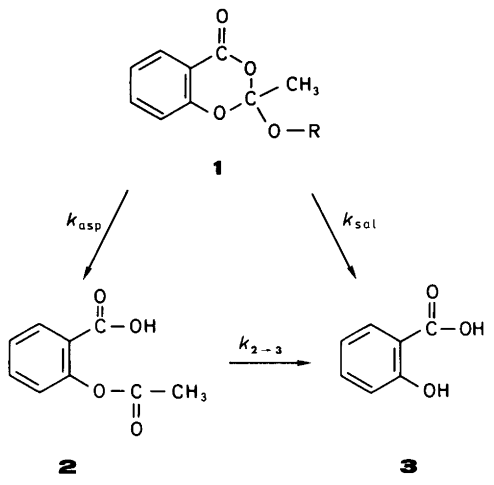

Fig. 1. Conversion of **1** into **2** or **3**. A variety of pathways are possible in the hydrolytic breakdown of **1**.[6]

In the past decade our laboratory has synthesized a number of orthoesters **1**.[5] However, the majority of our compounds have been subjected to non-enzymatic hydrolyses only. These early experiments demonstrated that only a few compounds **1** are hydrolysed to **2**, the majority to **3**, and some to both **2** and **3**. Hansen and Senning[6] found that **1e** was exclusively hydrolysed to **2** and therefore is a true *in vitro* aspirin prodrug, even though the half-life is relatively short. Ankersen and Senning[7] synthesized a series of benzyl orthoesters **4** with different substituents in the *ortho* positions of the phenyl moiety, but only the 2,6-dimethoxy compound formed **2** as the only hydrolysis product. As a follow-up to **1e** we decided to replace the *tert*-butyl moiety with other tertiary aliphatic alkyl groups. This paper outlines the synthesis and spectroscopic properties of fourteen orthoesters and hydrolysis results for the nine stable orthoesters **1**, all except **1f** and **1o** with a tertiary alkyl moiety. Hydrolysis experiments were performed in enzymatic as well as non-enzymatic solutions.

Results and discussion

Compounds **1a–1o** where prepared as described in synthesis I, eqn. (1), and II, eqn. (2), (originally developed by Rüchardt and Rochlitz[8] and Paris[9] *et al.*). As can be seen from Table 1, only compound **1b** was prepared by synthesis II. In general we preferred synthesis I because of the requirement in synthesis II of handling highly reactive trifluoroacetic anhydride. Published procedures employing less reactive and cheaper acid anhydrides[10] were not reproducible in our hands. Compounds **1e** and **1f** were originally prepared by Rüchardt and Rochlitz.[8]

* To whom correspondence should be addressed.

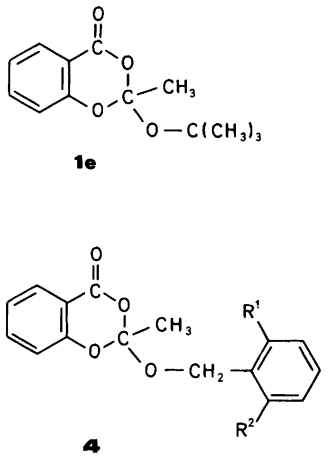

Fig. 2.

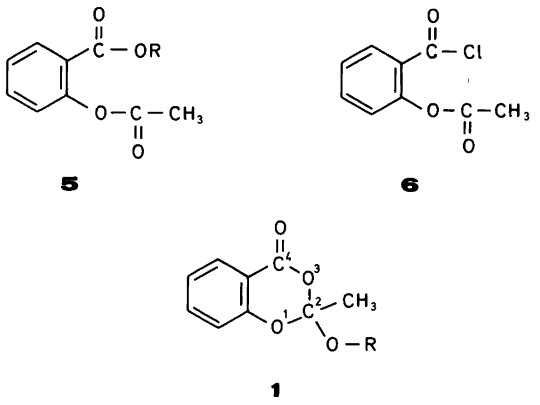

Fig. 3.

The formation of **1** and/or **5** from 2-acetoxybenzoyl chloride **6**, according to eqn. (1) depends in a complicated manner upon the presence or absence of base, the choice of solvent, and other conditions.[5] The fifteen compounds **1**

$$\mathbf{6} + \mathrm{ROH} \rightarrow \mathbf{1} + \mathbf{5} + \mathrm{HCl} \quad (1)$$

$$\mathbf{2} + (\mathrm{CF_3CO})_2\mathrm{O} + \mathrm{ROH} \rightarrow \mathbf{1} + \mathbf{5} + \mathrm{CF_3COOH} \quad (2)$$

exhibited a characteristic pattern in their NMR as well their IR spectra[11] (Table 2), different from that of the corresponding acyclic esters **4** (Table 2). Striking evidence for the structure of **1** is seen in NMR spectroscopy. The chiral carbon atom C(2), Fig. 3, when the substituent R is prochiral, as in **1a–1d**, **1g–1i**, **1l** and **1m**, renders two groups or atoms diastereotopic, i.e. spectroscopically non-equivalent,[12] which leads to a characteristic signal pattern. The chiral carbon atom C(2) of **1** has been rehybridized from sp^2 (in **2** and **6**) to sp^3, which moves the ^{13}C signal from typically, 155–180 ppm in a simple acetyl group to 112–114 ppm. In the ^1H NMR spectrum, the acetyl methyl signal moves from 2.05–2.20 ppm in the starting materials and related acyclic esters **4** to below 2.00 ppm (ca. 1.8 ppm) in the cyclic esters **1**. Only one carbonyl group is found in the cyclic orthoesters **1** and therefore only one carbonyl stretching absorption in IR (1760–1744 cm^{-1}).

Of the fifteen synthesized compounds **1** only nine were sufficiently stable for further hydrolysis experiments. Compound **1a** was stable, but we were unable to separate the cyclic ester from the corresponding acyclic ester **4**. As expected **1o** was quite lipophilic and therefore it was necessary to dilute the hydrolysis medium with an organic solvent. Table 3 shows results of the hydrolysis experiments.

The hydrolytic breakdown of **1** was found to follow pseudo-first-order kinetics. The observed rate constants,

Table 1. Preparation of **1**.

No.	R	Purity (%)	Time/h	Temp./°C	Yield (%)
a	2,2,2-Trichloroethyl	26	3.5	Reflux	20
b[a]	2,2,2-Trichloro-1,1-dimethylethyl	100			30
c	2,2-Dichloro-1,1-dimethylethyl	70	10.0	Reflux	43
d	2-Chloro-1,1-dimethylethyl	90	4.5	Reflux	60
e	*tert*-Butyl	95	4.0	Reflux	70
f[b]	*tert*-Butoxy	100	2.0	Amb.	80
g	1,1-Dimethyl-2-propenyl	85	1.5	Reflux	66
h	1-Methylcyclopentyl	90	2.5	Reflux	55
i	1-Methylcyclohexyl	70	2.8	Amb.	60
j	3-Ethyl-3-pentyl	50	6.0	Amb.	56
k	3-Methyl-3-hexyl	80	3.5	Reflux	61
l	2-(4-Chlorophenyl)-1,1-dimethylethyl	85	3.0	Amb.	56
m	1,1-Dimethyl-2-phenylethyl	60	2.0	Amb.	65
n	2-Methyl-2-adamantyl	65	2.0	Reflux	45
o	3β-Cholesteryl	95	24.0	Amb.	75

[a]Compound **1b** was prepared according to synthesis II; the commercial starting material 1,1,1-trichloro-2-methyl-2-propanol hemihydrate was dehydrated in toluene solution over $MgSO_4$ prior to use. All other compounds **1** were prepared according to synthesis I. [b]Prepared according to synthesis I, but without base.

Table 2. Physical properties of **1**.

No.	^{1}H NMR[a] δ_{CH_3} (ppm)	^{13}C NMR[a] $\delta_{C(2)}$ (ppm)	IR $\nu_{C=O}/cm^{-1}$	Other data
a	1.92	113.3	[b]	
b	1.93	113.2	1744 (KBr)	M.p. 86–87 °C, elem. analysis $C_{13}H_{13}Cl_3O_4$ (339.59)
c	1.82	112.9	1750 (film)	$n_D^{20.8}$ 1.5119
d	1.80	112.9	1752 (film)	$n_D^{20.8}$ 1.5289, elem. analysis $C_{13}H_{15}ClO_4$ (270.71)
e	1.80	113.2	1745 (film)	Elem. analysis $C_{13}H_{16}O_4$ (236.27)
f	1.89	113.1	1760 (film)	$n_D^{25.7}$ 1.4970, elem. analysis $C_{13}H_{16}O_5$ (252.27)
g	1.82	113.4	[c]	
h	1.69	112.9	[c]	
i	1.77	113.1	1744 (film)	Elem. analysis $C_{16}H_{20}O_4$ (276.33)
j	1.76	113.1	[c]	
k	1.80	113.3	1752 (film)	$n_D^{20.7}$ 1.4968
l	1.81	113.2	1752 (film)	
m	1.83	113.2	1752 (film)	$n_D^{20.9}$ 1.5239
n	1.84	113.4	[c]	
o	1.81	113.5	1755 (KBr)	M.p. 88–92 °C, $[\alpha]_D^{22}$ + 15.7° (*c* 0.01, C_6H_6) elem. analysis $C_{36}H_{52}O_4$ (548.80)

[a]In $CDCl_3$. [b]We were unable to separate **1a** from **5a**. [c]The compound was unstable and therefore no IR data was obtained.

k_{obs}, were calculated from the slopes of linear plots of the natural logarithms of either remaining **1** or increasing hydrolysis products against time. These experiments were performed by measurement of the alterations in UV absorption at 240 and 250 nm. The half-lives were then obtained from eqn. 3.

$$t_{1/2} = \ln 2/k_{obs} \quad (3)$$

In the case of mixed hydrolysis products (compounds **1c** and **1d**), the rate constant for the formation of **2**, k_{asp}, was simply obtained from eqn. (4).

$$k_{asp} = k_{obs} \times \% \text{ aspirin formed} \quad (4)$$

As seen in Table 3 we found four compounds, **1e**, **1k**, **1l** and **1m**, which exclusively form **2** in enzymatic as well as in non-enzymatic hydrolysis. Solutions containing 10 % human plasma give an adequate guideline for which products form upon enzymatic hydrolysis.[13] Unfortunately, we were unable to measure k_{obs} under enzymatic conditions, but it may be expected that $t_{1/2}$ decreases as the plasma content increases. Even though the above-mentioned four compounds comply with our definition of true aspirin prodrugs, the half-lives are still relatively short. A further improvement of **1l**, involving changes in the substitution pattern of the phenyl moiety, should be considered. On the other hand, the true salicylic acid prodrugs, compounds **1b** and **1f**, seem to be much more stable toward hydrolysis. In addition, 1,1,1-trichloro-2-methyl-2-propanol,[14,15] which is a well-known bactericide used as preservative in many drug preparations,[16,17] is released from **1b** upon hydrolysis. Because of these properties we found it worthwhile to carry out biological tests. It is well known that salicylic acid exerts a slight antiseptic and a considerable keratolytic action when used externally on the skin.[18,19] These proper-

Table 3. Hydrolysis data for the nine stable cyclic esters **1**.

No.	Hydolysis data in 10 % human plasma[c]	Hydrolysis data in 100 % aqueous phosphate buffer			
	Aspirin formed[d] (%)	k_{obs}/min^{-1}	$t_{1/2}$/min	Aspirin formed[d] (%)	k_{asp}/min^{-1}
b	0	0.0023	306.2	0	
c	31	0.0068	101.6	33	0.0023
d	30	0.0370	18.8	47	0.0174
e[a]	100		<1.0	100	
f	0	0.00006	9997.1	0	
k[a]	100		<1.0	100	
l	100	0.0491	14.1	100	
m	100	0.1550	4.5	100	
o[b]					

[a]The hydrolysis was too fast to detect. [b]Hydrolysed in THF–H_2O (1:1 v/v), the hydrolysis product was salicylic acid with $t_{1/2} > 48$ h. [c]In aqueous phosphate buffer. [d]The balance being **3**.

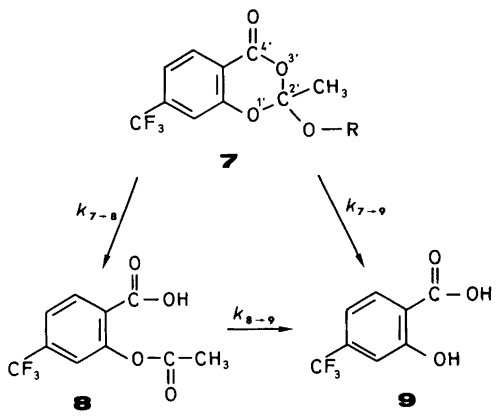

Fig. 4.

ties make it a beneficial agent in the local treatment of certain forms of eczematoid dermatitis (including psoriasis). Dr. K. Teelmann from Hoffmann–La Roche, Basel, Switzerland, kindly performed the biological tests. The two compounds were tested in a topical 'mouse papilloma model,' which is a model where some retinoids are able specifically to reduce the size of the treated papillomas, compared with the untreated papillomas. Both **1b** and **1f** were well tolerated, both locally and in general, but unfortunately no activity was found.

In parallel with **1** we synthesized a number of compounds **7**, Fig. 4, with the same alkyl substituents (R) as on **1**. Compound **8**, triflusal,[20,21] is a registered drug in Spain and is reported to inhibit platelet aggregation.[22,23] Distinct similarities were observed between the hydrolysis of **1** and **7** concerning $t_{1/2}$ and the relative amounts of the hydrolysis products **8** and **9**, compared with **2** and **3** (the results are to be published later).

Experimental

HPLC equipment. High-performance liquid chromatography (HPLC) was performed with a system consisting of a Kontron 420 HPLC pump, an ACS 750 UV detector operated at a fixed wavelength, and a Rheodyne 7125 injection valve with a 20 µl loop. A reversed phase column (150×4.6 mm), packed with Chromspher C18 (5 µm particles), initially connected to a guard column (Chromguard, 100×3 mm), was used. The kinetics were measured by means of a Kontron Uvikon 860 detector.

Identification. The identification of **1** was carried out by IR (Nicolet MX-S) and ^{1}H NMR and ^{13}C NMR spectroscopy (Varian Gemini, 200 MHz). Microanalyses were carried out by *Løvens Kemiske Fabrik,* DK-2750 Ballerup (Microanalytical Laboratory).

Synthesis I. A solution of 2-acetoxybenzoyl chloride[8] **6** (5 g, 25 mmol) in anhydrous chloroform was added dropwise, over a period of 15–30 min, to a solution of the appropriate alcohol (25 mmol) and triethylamine (2.5 g, 25 mmol), also in anhydrous chloroform. The mixture was then stirred under a nitrogen atmosphere until the starting materials could no longer be detected (i.e. by ^{1}H and ^{13}C NMR spectroscopy). If the reaction time is long (> 5 h) it is recommended that the temperature be raised to 50 °C or that the reaction be refluxed. The mixture was then washed with a saturated solution of $NaHCO_3$ (4×150 ml) and then dried over $MgSO_4$. After removal of the solvent the crude product was finally chromatographed on Al_2O_3.

Synthesis II. Trifluoroacetic anhydride (5.25 g, 25 mmol) was added to a stirred suspension of **2** (4.5 g, 25 mmol) in anhydrous toluene (100 ml). The reaction mixture was heated at 50 °C until a clear solution was obtained (4–5 min). The stirring was continued at room temperature for 30 min and then the alcohol (25 mmol) was added. The reaction mixture was stirred for 1 h at room temperature and cooled with an ice bath. To the cold solution was added a saturated solution of $NaHCO_3$ until the mixture was neutral or slightly basic. The organic layer was washed with water (3×150 ml) and brine (150 ml) and dried over $MgSO_4$. After removal of the solvent, the crude product was chromatographed on silica gel with petroleum ether–ether (4:1).

Hydrolysis in human plasma. The hydrolysis of the compounds in Table 3 was studied in 0.01 M phosphate buffer of pH 7.40, containing 10 % human plasma. The phosphate buffer was adjusted to a constant ionic strength (µ) of 0.5 by addition of the calculated amount of KCl. All solutions were kept at physiological temperature (37 °C). A 1×10^{-1} M stock solution in acetonitrile of **1** was prepared. The hydrolyses were initiated by addition of 50 µl of the stock solution to 5 ml plasma solution. At appropriate intervals samples of 600 µl were withdrawn and added to 1.2 ml of a 2 % solution of $ZnSO_4\cdot7\ H_2O$ in MeOH–H_2O (1:1 v/v) in order to deproteinize the plasma. Immediately after precipitation and centrifugation for 4–5 min at 5000 rpm, 20 µl of the clear supernatant were analyzed by reversed phase HPLC. To provide appropriate retention times and separation of the ester and hydrolysis products, the mobile phase system consisted of CH_3CN–CH_3OH–H_2O–H_3PO_4 (10:45:45:1 v/v). Phosphoric acid was added to suppress ionization of **2** and **3**. The flow rate was 1.0 ml min^{-1} and the column effluent was monitored at 215 nm.

Kinetic studies in aqueous buffer solution. Table 3 also shows the results of hydrolysis in 100 % phosphate buffer solution under the same conditions as previously mentioned. The products of the hydrolysis were quantified by measurement of the peak heights in relation to those of standards chromatographed under the same conditions.

The Topical Mouse Papilloma Model.[24] Mice were treated once with dimethylbenzanthracene followed by repeated applications of croton oil. Suitable mice, bearing at least 6 papillomas were selected for the experiments. Substances

(2.5 μl) to be investigated were applied at different concentrations to at least three papillomas each day, five times a week for a period of three weeks. In addition, a number of papillomas of the same animal were treated with the vehicle. Four were used per group (one concentration), each animal was kept in an individual cage. At weekly intervals, papilloma sizes were measured and the percentage regression recorded.

Acknowledgements. Grants from *Statens Lægevidenskabelige Forskningsråd, Lundbeckfonden, Aarhus Universitets Forskningsfond, Direktør Ib Henriksens Fond* and a scholarship from *Carlsbergfondet* are greatfully acknowledged, and we thank Dr. K. Teelmann, Hoffmann–La Roche, CH-4002 Basel, for performing the biological tests. Finally we thank *Skejby Sygehus* for donating the human plasma.

References

1. Rainsford, K. D. *Aspirin and the Salicylates,* Butterworths, London 1984.
2. Nielsen, K. K. *Dansk Kemi 70* (1989) 244.
3. Korolkovas, A. *Essentials of Medicinal Chemistry*, 2nd ed., Wiley–Interscience, New York 1988, p. 97.
4. McCormack, K. and Brune, K. *Arch. Toxicol. 60* (1987) 261.
5. Ankersen, M., Nielsen, K. K. and Senning, A. *Acta Chem. Scand. 43* (1989) 213.
6. Hansen, A. B. and Senning, A. *Acta Chem. Scand., Ser. B 37* (1983) 351.
7. Ankersen, M. and Senning, A. *Acta Chem. Scand. 43* (1989) 793.
8. Rüchardt, C. and Rochlitz, S. *Justus Liebigs Ann. Chem.* (1974) 15.
9. Paris, G. Y., Germaise, D. L., Cimon, D. G., Swett, L., Carter, G. W. and Young, P. *Med. Chem. 23* (1980) 79.
10. Harris Pharm. Ltd. *Belg. BE Pat.* 893,455 (1982); *Chem. Abstr. 98* (1983) 107308g.
11. Williams, D. H. and Fleming, I. *Spectroscopic Methods in Organic Chemistry*, 4th ed., McGraw–Hill, London 1987.
12. March, J. *Advanced Organic Chemistry*, 3rd ed., Wiley–Interscience, New York 1985.
13. Nielsen, N. M. and Bundgaard, H. *J. Med. Chem. 32* (1989) 727.
14. *Beilstein 1* E III, pp. 1586–1587.
15. *Merck Index,* 10th ed., No. 2098.
16. Camber, O. and Edman, P. *Int. J. Pharm. 39* (1987) 229.
17. Sim, A. T. R., White, M. D. and Denborough, M. A. *Int. J. Biochem. 19* (1987) 827.
18. Huber, C. and Christophers, E. *Arch. Dermatol. Res. 257* (1977) 293.
19. Nook, T. H. *Br. J. Dermatol. 117* (1987) 243.
20. Bara, E. F. and Moga, A. C. M. *U.S. Pat.* 4,096,252 (1978); *Chem. Abstr. 88* (1978) 89386t.
21. *Merck Index,* 10th ed., No. 9495.
22. Rutllant, M. L., Borell, M., Felez, J., Diaz, J. M., Vicente, J. M. and Garcia Rafanell, J. *Curr. Ther. Res. 22* (1977) 510.
23. Marti Masso, R., Rocha, E., Narvaiza, M. J. and Honorato, J. *Curr. Ther. Res. 25* (1979) 791.
24. Berenblum, J. and Shubik, P. *Br. J. Cancer 1* (1947) 383.

Received March 7, 1990.