Aspirin Prodrugs: 2-Methyl-2-aryloxy-4*H*-1,3-benzodioxin-4-ones Acting as True Aspirin Prodrugs

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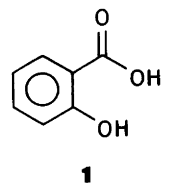
This paper is concerned with the synthesis, physical properties and both non-enzymatic and enzymatic *in vitro* hydrolysis of the title compounds **5**. Ten new and two known compounds **5** have been synthesized and characterized. *In vitro* enzymatic and non-enzymatic hydrolysis of ten compounds **5**, including the two known, revealed several of them to behave as true aspirin prodrugs.

Derivatives of salicylic acid (**1**) have been known for centuries. They have been used to treat fever and inflammation. In 1853 aspirin (**2**) was prepared by Gerhardt by acetylation of **1** with acetic anhydride. In modern terms **2** can be considered as prodrug of **1**. In the blood, liver, kidney and gastrointestinal tract **2** is hydrolysed to **1** and acetic acid by aspirin esterases.[1] Today **2** is also assumed to have effect on the acetylation of a variety of proteins in various tissues and components of the blood[1] and to inhibit prostaglandin and thromboxane biosynthesis.[2–7] The antipyretic effect and the analgesic effect of **2** can be explained by inhibition of a defined biochemical pathway responsible for the biosynthesis of the prostaglandins and related autocoids.[5] These autocoids are synthesized in many different organs and act locally.[6] They are not stored like neurotransmitters, but are continuously synthesized and immediately released into the circulation, where they are usually deactivated after only one passage through the lungs.[6]



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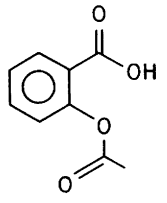

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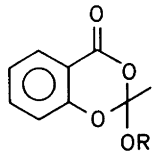

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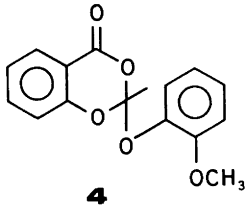

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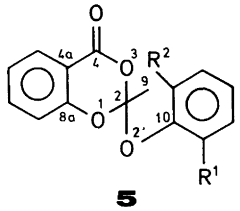

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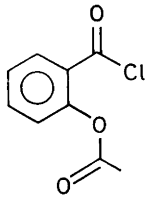

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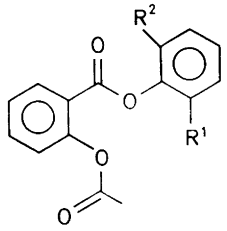

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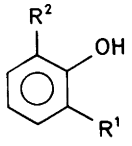

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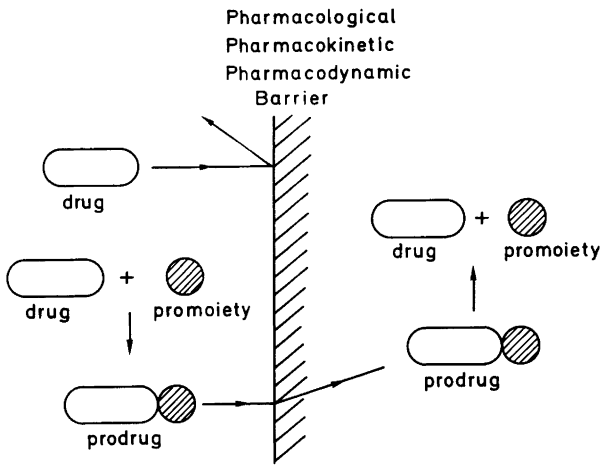

Fig. 1. The prodrug principle.

Compound **2** inhibits the conversion of arachidonic acid into the unstable endoperoxide intermediate, PGG_2, which is catalyzed by cyclooxygenase.[5,6]

The most common secondary effect of **2** is gastric irritation. This gastric damage can be brought about by at least two distinct mechanisms. Local irritation by the drugs in the stomach allows back-diffusion of acid into the mucosa and induces tissue damage. Even parenteral administration can cause gastric damage and bleeding. The predominant prostaglandins synthesized by the gastric mucosa are PGI_2 and PGE_2, which inhibit acid secretion by the stomach and promote the secretion of cytoprotective mucus in the intestine. Thus, inhibition of the biosynthesis of endogenous prostaglandins may render the stomach more susceptible to damage.[5]

The purpose of prodrugs can, among other things, be[6] (1) to increase the metabolic stability during the absorption and distribution phase, (2) to eliminate secondary effects, (3) to improve the smell and taste of the drug.

The prodrug principle can be illustrated as shown in Fig. 1. Prodrugs of pharmaceuticals consist of two parts, namely the drug and a promoiety. The prodrug itself must be pharmacologically inactive as must the promoiety. Furthermore, the prodrug must regenerate the original drug upon *in vivo* hydrolysis, oxidation, reduction, or some other metabolic conversion. The prodrugs of **2** include the ortho ester type compounds **3**. Up to mid-1988 fifty compounds of type **3** had been reported.[7]

Since the drug MR 693 (**4**, R = 2-methoxyphenyl) is apparently hydrolyzed both *in vitro* and *in vivo* to **2**,[8-10] we decided to investigate the merits of a variety of *ortho*-substitutions in the aryloxy part of **5**.

There have been suggested several synthetic pathways to **3**.[11-14] The method giving the best results is the one of Rüchardt and Rochlitz[11] in which the ortho esters **3** are prepared from *O*-acetylsalicylic acid chloride **6** and the appropriate alcohol. In all procedures described so far, the ester **7** is formed together with the cyclic isomer **3**.

As shown in Fig. 2 the hydrolysis can follow various pathways. The compounds prepared by us have been studied in detail. Their synthesis, physical properties and behavior upon enzymatic and non-enzymatic *in vitro* hydrolysis in buffered 10% human plasma and aqueous phosphate buffer, i.e. their potential as aspirin prodrugs, are discussed below.

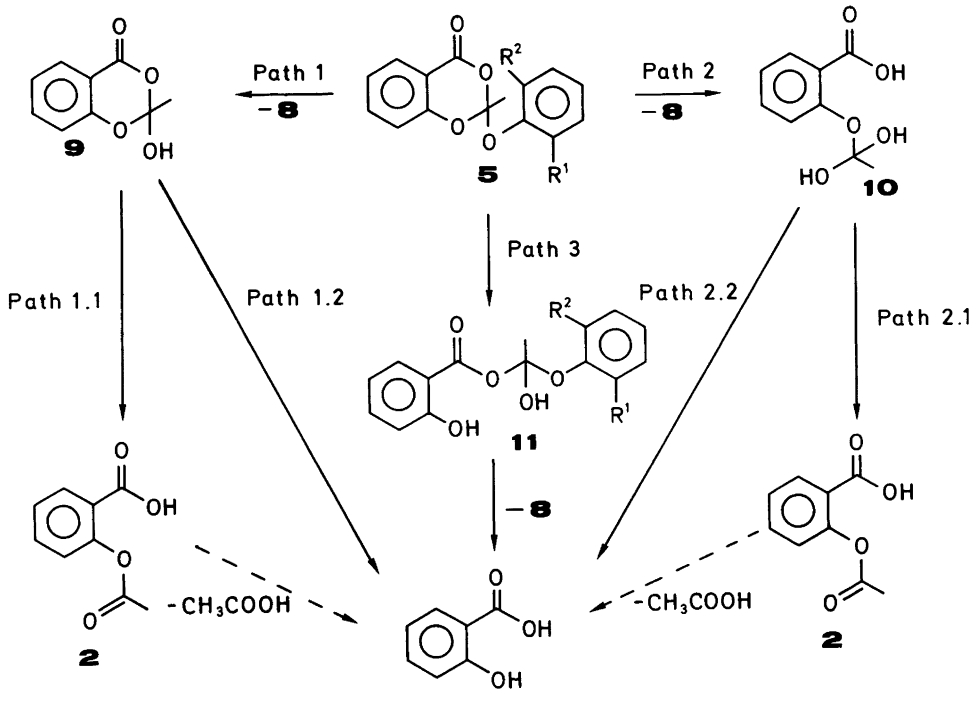

Fig. 2. The hydrolysis of **5**.

Table 1. Preparation of **5** and **7**.

Compound	Substituents (R^1, R^2)	Ratio **5**:**7**[a] Method				Purity of **5**[b] (%)	Yield of **5** (%)
		I	II	III	IV		
5-1/7-1	Cl,Cl		5:1			100	76
5-2/7-2	Br,H		7:2			100	75
5-3/7-3	Cl,H[c]		4:1			100	49
5-4/7-4	OCH_3,H[c]	13:2	1:2	0:1	3:2	50	50
5-5/7-5	$COOCH_3$,H	0:1	–[d]	–[d]			
5-6/7-6	CH_2CH_3,H	1:10	8:1			100	74
5-7/7-7	CH_3,CH_3		1:1			30	30
5-8/7-8	OCH_2CH_3,H		8:1			100	44
5-9/7-9	OCH_3,OCH_3	3:1	9:1			100	83
5-10/7-10	$CH(CH_3)_2$,H	1:5	12:1			93	70
5-11/7-11	CH_3,CH_3,CH_3[e]	0:1		1:0		95	91
5-12/7-12	$OCH(CH_3)_2$,H	0:1	0:1	10:1		100	86
5-13/7-13	$C(CH_3)_3$,H	2:1	14:1			93	53

[a]The ratio **5**:**7** was determined by evaluation of the NMR spectra of the crude products. [b]The purity was determined by NMR spectroscopy. 100 % means that no other compounds were detectable. [c]The CAS registry numbers of **5**-3 and **5**-4 are [52602-06-9] and [81674-79-5], respectively. [d]Neither of the compounds **5**-5 or **7**-5 was formed. [e]The phenol used was 2,4,6-trimethylphenol.

Table 2. Physical properties of 2-methyl-2-aryloxy-4*H*-1,3-benzodioxin-4-ones **5**.

Compound	Formula	M.p.[a]/°C	^{1}H NMR[b] δ_{CH_3} (ppm)	^{13}C NMR[b] $\delta_{C(2)}$ (ppm)	^{13}C NMR[b] $\delta_{C(9)}$ (ppm)	IR $\nu_{C=O}$/cm^{-1}	UV λ_{max}/nm
5-1	$C_{15}H_{10}Cl_2O_4$	107.4–108.4	1.92	113.92	23.15	1752[c]	300[d]
5-2	$C_{15}H_{11}BrO_4$	100.6–102.7	1.93	113.96	23.12	1744[c]	297[e]
5-3	$C_{15}H_{11}ClO_4$	84.5–85.0	1.92	113.91	23.05	1752[c]	300[d]
5-4	$C_{16}H_{14}O_5$	40–43[f]	1.96	114.30	23.27	1752[c]	300[d]
5-6	$C_{17}H_{16}O_4$	64.1–69.5	1.90	114.12	23.30	1752[c]	298[e]
5-7	$C_{17}H_{16}O_4$	Oil	1.72	113.96	–[h]	–[h]	299[d]
5-8	$C_{17}H_{16}O_5$	69.3–70.4	1.92	114.21	23.28	1736[c]	298[e]
5-9	$C_{17}H_{16}O_6$	137.4–140.5	1.97	114.68	23.93	1752[c]	297[e]
5-10	$C_{18}H_{18}O_4$	Oil	1.82	114.12	23.22	1760[g]	300[e]
5-11	$C_{18}H_{18}O_4$	Oil	1.76	113.96	23.27	1750[g]	299[e]
5-12	$C_{18}H_{18}O_5$	43.0–45.6	1.91	114.38	23.20	1760[c]	297[e]
5-12	$C_{19}H_{20}O_4$	Oil	1.99	114.01	–[h]	1752[g]	–[h]

[a]The compounds were recrystallized from petroleum ether. [b]Solvent: $CDCl_3$. [c]In KBr. [d]Solvent: ethanol. [e]Solvent: acetonitrile. [f]The compound isolated was a 1:1 mixture of **5**-4 and **7**-4. [g]Film. [h]Not determined.

Table 3. Physical data for **7**.

Compound	Formula	M.p.[a]/°C	^{1}H NMR[b] δ_{CH_3} (ppm)	^{13}C NMR[b] $\delta_{C(2)}$ (ppm)	^{13}C NMR[b] $\delta_{C(9)}$ (ppm)
7-1	$C_{15}H_{10}Cl_2O_4$	Oil	2.31	–[d]	–[d]
7-2	$C_{15}H_{11}BrO_4$	Oil	2.30	–[d]	–[d]
7-3	$C_{15}H_{11}ClO_4$[c]	Oil	2.30	–[d]	–[d]
7-4	$C_{16}H_{14}O_5$[c]	66.2–68.1	2.28	170.34	21.24
7-5	$C_{17}H_{14}O_6$[c]	82.2–83.5	2.27	170.11	21.07
7-6	$C_{17}H_{16}O_4$	58.3–61.8	2.29	170.19	21.07
7-7	$C_{17}H_{16}O_4$	Oil	2.28	–[d]	–[d]
7-8	$C_{17}H_{16}O_5$	Oil	2.25	–[d]	–[d]
7-9	$C_{17}H_{16}O_6$	Oil	2.27	–[d]	–[d]
7-10	$C_{18}H_{18}O_4$	83.2–84.0	2.29	170.25	21.08
7-11	$C_{18}H_{18}O_4$	104.8–106.5	2.27	170.22	21.10
7-12	$C_{18}H_{18}O_5$	103.2–106.8	2.30	170.70	21.07
7-12	$C_{19}H_{20}O_4$	Oil	2.30	–[d]	–[d]

[a]The compounds were recrystallized from petroleum ether. [b]Solvent: $CDCl_3$. [c]The CAS registry numbers of **7**-3, **7**-4 and **7**-5 are [52602-89-8], [55482-89-8], and [85531-21-1], respectively. [d]Not determined.

Results and discussion

As mentioned before, this investigation only concerns compounds which contain an *o*-substituted aryloxy moiety. Table 1 shows the compounds which have been synthesized. Most of them were prepared according to general synthesis II *(vide infra)*. None of the compounds **5** could be conveniently prepared according to general synthesis I *(vide infra)*. Compounds **5**-11 and **5**-12 were obtained according to general synthesis III *(vide infra)*. They were essentially pure even before recrystallization from light petroleum. Tables 2 and 3 show the physical properties of compounds **5** and **7**, some of which could only be obtained as inseparable **5/7** mixtures.

The strongest argument for the identity of **5** and **7** is found in the NMR data. Fig. 3 shows a typical ^{1}H NMR spectrum of a cyclic isomer **5** versus one of an acyclic isomer **7**. As can be seen from Tables 2 and 3, the *C*-methyl signal moves from 2.25–2.31 ppm in the esters **7** to 1.72–1.99 ppm in the ortho esters **5**, cf. Refs. 12, 14 and 15. In the ^{13}C NMR spectrum the carbonyl C atom of the acetyl group of **7** gives rise to a signal at 170–171 ppm while the corresponding sp^3-C(2) of **5** resonates at 113–115 ppm.[14,16,17] In addition, the *C*-methyl signal moves from 21 ppm in **7** to 23–24 ppm in **5**. Fig. 4 shows the ^{13}C NMR spectrum of **5**-9. Another significant difference between compounds **5** and **7** can be seen in their carbonyl stretching mode in IR spectroscopy. The esters **7** have two carbonyl groups with correspondingly separate absorptions while the esters **5** have only one.

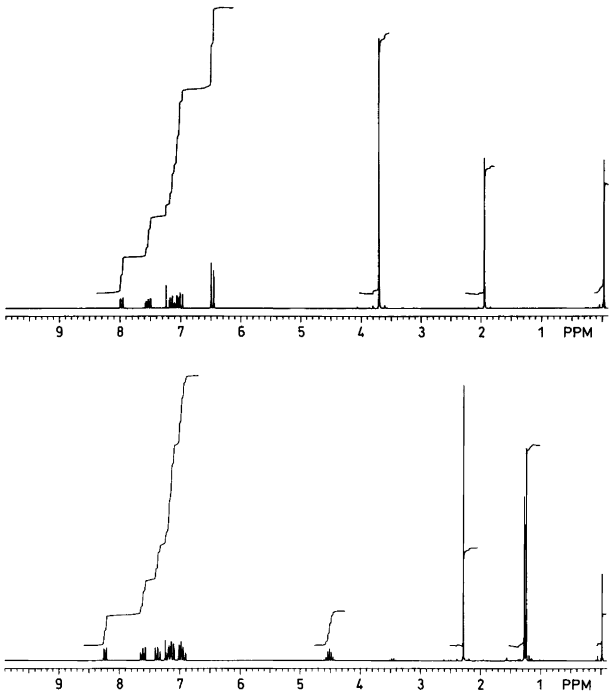

Fig. 3. ^{1}H NMR spectrum of **5**-9 (above) and **7**-12. Note the methyl signals.

Hydrolysis. Table 4 shows the pseudo-first-order rate constants for the hydrolysis of **5**. The rate constants were calculated from the slopes of the plots of the logarithm of **5** concentration or of the concentration of **1** and/or **2** against time. The concentrations were measured by UV absorption.

The amounts of **1** and/or **2** formed in the hydrolysis experiments were determined by HPLC analysis of aliquots. Compounds **5**-4, **5**-8 and **5**-9 were studied over a wide range of pH. The buffers used were phosphate, acetate and borate, respectively. The data are given in Table 5. The other compounds were not investigated, but they are assumed to follow the same pattern. The uncertainties of the data so obtained were calculated by a standard procedure.[19]

Conclusions

As can be seen from Table 4 all compounds **5**, except **5**-2, regenerate **2** or **2/1** upon both enzymatic and non-enzymatic *in vitro* hydrolysis. Furthermore, several compounds **5** have relatively long half-lives and their hydrolyses are only slightly dependent on pH. Compounds **5**-4 and **5**-9 especially act as exclusive aspirin prodrugs. It should be

Table 4. In vitro hydrolysis of compounds **5** at pH 7.4.

Compound	Aqueous phosphate buffer		10 % Buffered human plasma	
	Aspirin formed (%)	$t_{1/2}$/min	Aspirin formed (%)	$t_{1/2}$/min
5-1	99±7	<0.5	64±4	<0.5
5-2	5±1	17.3±2.9	0±1	10.4±0.7
5-3	58±3	9.9±1.3	84±6	4.6±0.6
5-4	88±6	138.6±15.9	68±4	80.3±8.8
5-6	92±7	19.3±5.1	76±5	12.2±1.3
5-8	68±4	24.3±3.0	78±5	7.2±0.4
5-9	99±7	54.9±1.8	77±5	15.0±2.5
5-10	100±7	20.1±1.5	94±7	10.9±3.0
5-11	67±4	17.7±0.6	23±1	2.0±0.2
5-12	84±6	47.5±4.8	7±1	8.4±1.9

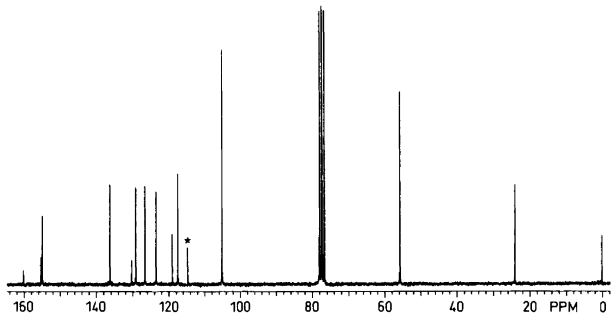

Fig. 4. ^{13}C NMR spectrum of **5**-9. The small peak marked * is essential for the recognition of **5**.

noticed that until now **5**-4 has never been proved to act as a true prodrug, although clinical tests are being carried out.[20] The nature of the *ortho*-substituent seems not to be important for the hydrolytic breakdown, i.e. whether **1** or **2** will be formed.

Experimental

HPLC equipment. HPLC was carried out with a Kontron apparatus consisting of an LC pump 420, an ASC 750 UV detector operating at a fixed wavelength (215 nm), a Rheodyne injection valve with a 20 µl loop and a Chrompack column (150×4.6 mm) packed with Chromspher C18 (5 µm particles), initially connected to a Chromguard column (100×3 mm).

Analyses. The identification of the compounds **5** and **7** was carried out mainly by ^1H NMR and ^{13}C NMR spectroscopy (Varian Gemini 200) together with IR (Nicolet MX-S), UV (Kontron Uvikon 860) and MS (Micromass 7070F). Elemental analyses were performed by *Løvens Kemiske Fabrik*, DK-2750 Ballerup (Microanalytical Laboratory).

Kinetics. The kinetics were carried out by HPLC. The *in vitro* hydrolytic breakdown of the aspirin derivatives **5** was studied in a 0.01 M aqueous phosphate buffer and 10% buffered human plasma at 37 °C, pH 7.4 (adjusted with 2 M sodium hydroxide solution) and $\mu = 0.5$ (adjusted with potassium chloride). The buffers of pH 3.0, 4.0, 5.0, 6.0, 6.9, 9.0 and 10.0 were adjusted with 2 M sodium hydroxide or 2 M hydrogen chloride. They were 0.01 M and $\mu = 0.5$. The progress of the hydrolysis was followed by use of reversed-phase HPLC procedures. Three different mobile phase systems were used in order to separate **5**, **2** (**1**), and **8**, namely $CH_3OH-CH_3CN-H_2O-H_3PO_4$ (45:10:45:1 v/v), $CH_3OH-CH_3CN-H_2O-H_3PO_4$ (30:10:60:1 v/v) and $CH_3OH-CH_3CN-H_2O-H_3PO_4$ (20:10:70:1 v/v).

In the case of the compounds **5**-4, **5**-9, **5**-10 and **5**-11 the last system was used. For the other compounds, the second system was used. For the ortho ester detection, i.e. retention time and calculation of rate constants, the first system was used. The flow rate was 1 ml min^{-1}. The hydrolysis was initiated by the addition of 50 µl of a stock solution of the compound **5** in acetonitrile to 5 ml preheated buffer, the final concentration of **5** being about 10^{-4} M. At appropriate intervals 20 µl samples were chromatographed by HPLC.

General synthesis I (with base).[7] To a solution of 0.025 mol *o*-substituted phenol **8** and 2.53 g (0.025 mol) triethylamine in chloroform were added 4.95 g (0.025 mol) **6**,[11] dissolved in chloroform. The solution was then refluxed for 2–4 h under a stream of nitrogen before it was washed 3–4 times with a 0.5 M sodium hydrogen carbonate solution. The organic phase was filtered through Al_2O_3 to remove remaining starting material. The filtrate was dried over magnesium sulfate and the solvent evaporated under reduced pressure to yield an oil, containing **5** and **7** in a ratio that varied from case to case. Usually, compound **5** could be separated by the addition of petroleum ether, diethyl ether or a mixture of these.

General synthesis II (without base).[7] This method is similar to I, but without base.

General synthesis III.[7] A suspension of 4.50 g (0.025 mol) **2** and 40 ml benzene was heated at 45 °C for 15 min. Then 5.25 g (0.025 mol) trifluoroacetic acid anhydride were

Table 5. pH Profile for compounds **5**-4, **5**-8 and **5**-9.

pH	**5**-4		**5**-8		**5**-9	
	Aspirin formed (%)	$t_{1/2}$/min	Aspirin formed (%)	$t_{1/2}$/min	Aspirin formed (%)	$t_{1/2}$/min
3.0	87±6	66.0±14.3	73±5	15.0±1.4	95±7	40.8±1.8
4.0	84±6	82.1±17.5	72±4	29.9±3.1	93±7	48.3±2.1
5.0	84±6	79.4±18.4	68±4	22.6±4.3	96±7	51.8±4.8
6.0	83±5	76.1±3.7	67±4	22.6±3.9	95±7	60.9±6.8
6.9	89±6	120.1±15.0	70±4	24.1±3.2	90±6	68.5±12.4
7.4	83±6	138.6±15.9	68±4	24.3±3.0	99±7	54.9±1.8
9.0	53±3	61.8±13.1	73±5	20.3±0.5	76±5	21.7±0.3
10.0	25±1	36.6±0.2	60±3	9.9±0.7	23±1	22.5±0.9

added and the mixture left for 30 min at room temperature. Then 0.025 mol **8** in benzene were added dropwise and the preparation continued as described in I.

General synthesis IV.[7] A solution of 0.025 mol **8** and 2.53 g (0.025 mol) triethylamine was added dropwise to 2.7 g (0.025 mol) trimethylsilyl chloride and refluxed for 1–2 h. A solution of 4.95 g (0.025 mol) **6** in chloroform was then added dropwise. After 2–3 h at reflux temperature, the preparation was continued as described in I.

If **5** and **7** could not be separated at once the mixture was dissolved in chloroform, filtered through Al_2O_3, and the filtrate evaporated. To the viscous liquid so obtained was added a small amount of light petroleum, diethyl ether or a mixture of these. This procedure was repeated several times if crystallization did not occur at once.

The new compounds **5** and **7** as well as the known compounds gave satisfactory elemental analyses. Detailed descriptions of the preparations for each compound are available on request.[21]

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References

1. Rainsford, K. D. *Aspirin and the Salicylates,* Butterworths, London 1984.
2. Vane, J. R. *J. Allergy Clin. Immun. 58* (1976) 691.
3. Vane, J. R. *Agents Actions 8* (1978) 427.
4. Flower, R. J. and Blockwell, G. J. *Nature (London) 278* (1979) 456.
5. Flower, R. J., Moncada, S. and Vane, J. R. In: Goodman, L. S. and Gilman, A., Eds., *The Pharmacological Basis of Therapeutics,* 7th ed., MacMillan, New York 1984, p. 674.
6. Nogrady, T. *Medicinal Chemistry,* 2nd ed., Oxford University Press, New York 1989.
7. Ankersen, M., Nielsen, K. K. and Senning, A. *Acta Chem. Scand. 43* (1989) 213.
8. Harris Pharmaceuticals Ltd. *Belg. Pat.* 893,455 (1982); *Chem. Abstr. 98* (1983) P107308g.
9. Zambeletti, Dr. L., S. p. A. *Belg. Pat.* 890,731 (1982); *Chem. Abstr. 96* (1982) P199707w.
10. Quadro, G. *Eur. Pat. Appl.* 80,609 (1983); *Chem. Abstr. 99* (1983) P175779g.
11. Rüchardt, C. and Rochlitz, S. *Liebigs Ann. Chem.* (1974) 15.
12. Paris, G. Y., Germaise, D. L., Swett, L., Carter, G. W. and Young, P. *J. Med. Chem. 23* (1980) 79.
13. Fried, J. *US Pat.* 3,432,830 (1968); *Chem. Abstr. 70* (1969) P68386f.
14. Hansen, A. B. and Senning, A. *Acta Chem. Scand., Ser. B 37* (1983) 351.
15. Williams, D. H. and Fleming, I. *Spectroscopic Methods in Organic Chemistry,* 4th ed., McGraw-Hill, New York 1987.
16. Akhrem, A. A., Zherkov, V. V., Zaitseva, G. V. and Mikhailopulo, I. A. *Tetrahedron Lett.* (1973) 1475.
17. Fried, J. and Elliott, W. J. *J. Org. Chem. 73* (1978) 2708.
18. Renson, M. and Schoofs, F. *Bull. Soc. Chim. Belg. 69* (1960) 326.
19. Barfod, N. C. *Experimental Measurements: Precision, Error and Truth,* Addison–Wesley, London 1976, p. 56.
20. Quadro, G. *Personal communications.*
21. Hundewadt, M. *Cand. Scient. Thesis,* Department of Chemistry, University of Aarhus, Aarhus, Denmark. *In preparation.*

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