the xenograft systems, some synthetic modifications in the structure of aframodial are currently carried on for subsequent testings by the NCI and these results will be reported later.

Experimental

1. General experimental procedures

M.p's. were determined on a Gallenkamp apparatus and are not corrected. TLC analyses were carried out on 0.25 mm thick precoated silica plates (Merck Fertigplatten Kieselgel 60 F_{254}) with the mobile phase hexane/EtOAc 85 : 15. TLC spots were visualized under UV light and preferentially by 50% sulfuric acid spray and subsequent heating (black spots). CC was carried out on Merck Kieselgel 60 (70–230 mesh). NMR spectra were recorded on a JEOL JMN EX 400 spectrometer in ~5% solution at 25 °C. IR by a Mattson Polaris FTIR spectrometer in the solid state (KBr).

2. Plant material

The seeds of A. daneillii were collected in Mbalmayo in the Centre Province of Cameroon in February 1997 and identified by Dr Sonke. A voucher specimen of this plant has been deposited in the National Herbarium, Yaounde.

3. Extraction and isolation

Dried seeds (1 kg) of A. daneillii were powdered and extracted with hexane in a Soxhlet apparatus for 24 h. The hexane extract was concentrated and the residue (89.0 g) was first flash-chromatographed on a silica gel pad. Fractions obtained when eluting with Hexane/EtOAc 95 : 5 gave pure aframodial (**1**, 220 mg), galanolactone (**2**, 68 mg) and galanal B (**3**, 72 mg).

Compound **1** (colourless needles): m.p. 91–92 °C; ^{13}C NMR δ (^{13}C): 39.56 (C1); 18.34 (C2); 41.88 (C3); 33.64 (C4); 52.17 (C5); 19.88 (C6); 39.56 (C7); 57.51 (C8); 55.22 (C9); 40.03 (C10); 22.41 (C11); 161.18 (C12); 136.05 (C13); 36.01 (C14); 198.00 (C15); 194.07 (C16); 48.81 (C17); 33.64 (C18); 21.62 (C19); 14.81 (C20).

Compound **2** (colourless needles): m.p. 125–126 °C; ^{13}C NMR δ (^{13}C): 39.19 (C1); 18.67 (C2); 41.77 (C3); 33.40 (C4); 52.42 (C5); 19.93 (C6); 35.66(C7); 57.36 (C8); 55.01 (C9); 39.56 (C10); 39.56 (C11); 142.80 (C12); 124.70 (C13); 25.24 (C14); 65.42 (C15); 171.06 (C16); 48.66 (C17); 33.39 (C18); 21.62 (C19); 14.83 (C20).

Compound **3** (colourless needles): m.p. 134–135 °C; δ (^{13}C): 38.96 (C1); 18.73 (C2); 41.77 (C3); 33.46 (C4); 55.58 (C5); 19.15 (C6); 34.42 (C7); 55.42 (C8); 55.70 (C9); 38.96 (C10); 24.20 (C11); 157.92 (C12); 140.89 (C13); 28.74 (C14); 78.86 (C15); 193.72 (C16); 208.31 (C17); 33.36 (C18); 21.44 (C19); 15.96 (C20).

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Analgetic properties of glycosyl diclofenac derivatives

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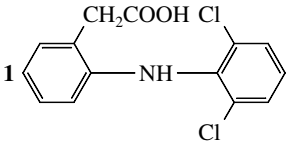
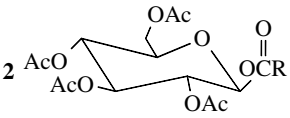
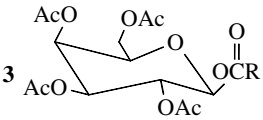
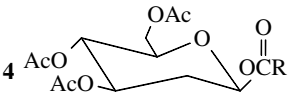
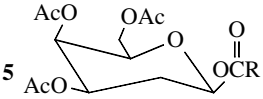
Diclofenac, natrium salt of *o*-(2,6-dichloroanilino) phenylacetic acid (**1**, as free acid) has been widely used in therapy for over 20 years as a powerful nonsteroid antiinflammatory drug (NSAID) [1, 2]. Similarly to other NSAID, it causes numerous adverse effects in the gastrointestinal system such as: nausea, vomiting, abdominal pain and also leads to the formation of ulcers [3]. There are at least two distinct mechanisms of gastric damage by these agents: the inhibition of the synthesis of prostaglandins PGE2 and PGI2, which are protective towards the stomach mucosa, and the direct irritation of the mucosa [4], which depends on the chemical structure of the drug, and in particular, on the presence of a carboxyl group. In order to decrease the irritating effect of this compounds, attempts have been made to hide the carboxyl group [5–7]. Hussain [8] obtained a sugar derivative of aspirin and he concluded that, at pH values between 3 and 9, aspirin is released from this prodrug by hydrolysis. In numerous laboratories the synthesis of derivatives of **1** is conducted as such compounds are expected to have smaller ulcerogenic properties e.g. In fact, methyl esters of **1** showed less ulcerogenic activity [7]. We obtained several glycosyl derivatives of acid **1** as potential prodrugs [9]. Preliminary *in vitro* experiments on these derivatives (by means of the polarymetric method) showed that in 0.1N HCl, acid **1** is released [10]. We also found that the release of this compound may take place at pH values between 3 and 7 [11]. In this work we studied the analgetic activity of glycosyl derivatives of acid **1** containing glucose **2**, galactose **3**, 2-deoxy-glucose **4** and 2-deoxy-galactose **5**. The acute toxicity of the new diclofenac derivatives **2**–**5** after intraperitoneal administration was found to be about 2.5–3 times lower than that of diclofenac as free acid (**1**) (Table 1). The glucose derivative (**2**) at doses of 0.1 and 0.5 ALD_{50} showed analgetic effects both in the hot plate (Table 1) and tail flick (Table 2) tests. After administration of the galactose derivative **3** in doses of 0.1 and 0.5 ALD_{50}, the reaction lag time to pain was also longer in the tail flick test, and was seen after 120 min of the observation (Table 2). In the hot plate test this effect was already present 60 min after administration of the drug at a dose of 0.1 ALD_{50} (Table 1). The 2-deoxy-glucose derivative **4** showed a weaker analgesic effect, whereas the 2-deoxy-galactose derivative **5** did not show any effect (Tables 1 and 2). Diclofenac as free acid (**1**) showed analgetic effects in both tests and this effect was stronger at the higher dose in the hot plate test (Table 1). A comparison of the analgetic effects of the new sugar derivatives of diclofenac **2**–**5** and **1** showed that only the glucose derivative **2** had an effect in the tail flick test similar to that of **1** at both concentrations tested. In the hot plate test the effect of **2** is similar to that of **1** at a dose of 0.1 ALD_{50}, whereas at 0.5 ALD_{50}, it was smaller. This may be associated with the surplus of the substrate for the reaction of disintegration which takes place.

In the near future larger scale pharmacological studies on the glucose derivative **2** will be conducted.

Table 1: Approximate values of LD_{50} (ALD_{50}) and analgetic effect of glycosyl derivatives of diclofenac in the hot plate test

Compd.	Dose i.p. (ratio of ALD_{50})	ALD_{50} (g/kg i.p.)	Time of reaction (s) after		
			1 h	1.5 h	2 h
Methylcellulose (control group)	0.1 ml/10 g		12.1 ± 0.4	12.5 ± 0.6	11.8 ± 0.5
1 	0.1 0.5	0.6	16.0 ± 1.4 a)* 35.2 ± 4.5 a)***	18.2 ± 1.5 a)** 29.3 ± 3.9 a)***	15.7 ± 1.3 a)* 25.3 ± 2.3 a)***
2 	0.1 0.5	>2	18.7 ± 0.89 a)*** 16.1 ± 1.2 a)**; b)***	17.0 ± 1.1 a)** 11.9 ± 1.0 b)***	8.0 ± 1.4 a)*** 8.4 ± 0.3 a)***; b)***
3 	0.1 0.5	1.5	16.1 ± 1.4 a)* 17.0 ± 0.6 a)***; b)***	11.5 ± 1.1 b)** 12.1 ± 1.1 b)***	15.3 ± 1.4 a)* 12.1 ± 0.6 b)***
4 	0.1 0.5	>2	10.9 ± 0.8 b)** 13.9 ± 2.2 b)***	14.7 ± 1.0 16.0 ± 1.8 b)**	12.5 ± 0.88 18.1 ± 2.1 a)**; b)*
5 	0.1 0.5	>2	12.3 ± 1.0 b)* 10.3 ± 1.0 b)***	12.7 ± 0.4 b)*** 13.0 ± 1.5 b)**	14.3 ± 0.6 a)**; b)* 11.1 ± 1.0 b)***

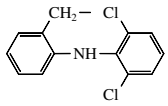
Mean values and SEM are given; statistically significant differences at $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ (Student t test)
[a] Compared to methylcellulose group; [b] Compared to diclofenac group; R = 

Table 2: Analgetic effect of glycosyl derivatives of diclofenac in the tail flick test

Compd.	Dose i.p. (ratio of ALD_{50})	Time of reaction (s) after		
		1 h	1.5 h	2 h
Methylcellulose (control group)	0.1 ml/10g	13.7 ± 0.3	11.3 ± 0.8	12.8 ± 1.0
1	0.1	16.4 ± 1.1 a)*	17.0 ± 1.8 a)*	17.5 ± 1.6 a)*
	0.5	20.4 ± 2.2 a)***	14.9 ± 2.5	15.0 ± 2.3 a)*
2	0.1	21.4 ± 1.7 a)***; b)*	15.4 ± 1.8 a)*	15.6 ± 3.1 a)*
	0.5	15.0 ± 0.4 a)*; b)*	19.3 ± 1.6 a)***	12.3 ± 0.6
3	0.1	14.4 ± 1.7	13.1 ± 0.8	15.0 ± 1.2 a)*
	0.5	16.3 ± 1.9	14.5 ± 1.4	14.1 ± 1.0
4	0.1	19.1 ± 2.3 a)*	14.3 ± 0.9	12.9 ± 0.9 b)*
	0.5	17.0 ± 1.3	15.5 ± 1.9	14.0 ± 2.5
5	0.1	13.6 ± 1.4 b)**	14.5 ± 1.2 a)*	13.8 ± 1.1 b)*
	0.5	13.1 ± 1.3	12.1 ± 2.2	10.6 ± 1.4

Mean values and SEM are given; statistically significant differences at $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ (Student t test)
[a] Compared to methylcellulose group; [b] Compared to diclofenac group

Experimental

1. Chemistry

The ^{1}H and ^{13}C NMR spectra were obtained with a Bruker AC 200 spectrometer (200.11 MHz, 50.33 MHz, 81.01 MHz respectively, $CDCl_3$) using tetramethylsilane (TMS) as the internal standard. Derivatives of the **2**–**4** were prepared according to a procedure recently described [9]. By means of this method using α-dithiophosphate of 2-deoxy-D-galactose [12] in stoichiometric amounts with acid **1** in CH_2Cl_2 at RT (48 h) in the presence of silver carbonate we obtained 1-*O*-[*o*-(2,6-dichloroanilino)phenylacetyl]-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactose (**5**). By crystallization from EtOH **5** was isolated in 87% yield, m.p. 139–140 °C (colourless crystals), $[\alpha]_{578}^{29} = -2.8$ (c = 0.14 in $CHCl_3$). ^{1}H NMR: δ (ppm) = 1.61 (s, 1 H, NH), 1.81–1.99 (m, 1 H, 2-H_{ax}) 2.02, 2.04, 2.07 (3s, 9 H, 3 Ac), 2.32–2.42 (m, 1 H, 2-H_{eq}), 3.69–3.79 (m, 1 H, 5-H), 3.87 (d, 2 H, CH_2), 4.07 (dd, $J_{5,6b} = 2.3$ Hz, $J_{gem} = 12.4$ Hz, 1 H, 6_b-H), 5.0–5.08 (m, 2 H, 3-H, 4-H), 5.84 (dd, $J_{1,2a} = 9.9$ Hz, $J_{1,2e} = 2.3$ Hz, 1 H, 1-H), 6.54–7.35 (m, 7 $H_{arom.}$). ^{13}C NMR: δ (ppm) = 20.53 (s, 2 CH_3CO), 20.64 (s, CH_3CO), 34.48(s, C-2),

38.13 (s, CH_2), 61.79 (s, C-6), 68.09, 69.82, 72.74 (3s, C-3 to C-5); 91.60 (s, C-1), 118.54, 122.17, 123.70, 123.95, 128.10, 128.70, 129.33, 130.78, 137.66, 142.56 (10s, $C_{arom.}$), 169.52 (s, CH_2OCO), 169.80, 170.04, 170.43 (3s, CH_3CO).

2. Pharmacology

2.1. Animals and treatment

The experiments were carried out on Swiss male mice (18–24 g). The mice were kept in group cages under laboratory conditions at a temperature of 20–21 °C, natural day/night cycle and they had free access to commercial chow food and water. All experiments were performed between 11.00 a.m. and 02.00 p.m. The compounds suspended in 1% solution of methylcellulose were administered intraperitoneally in doses of 0.1 and 0.5 ALD_{50}, 0.1 ml/10 g b.w.

2.2. Acute toxicity

Approximate values of LD_{50} (ALD_{50}) were determined by Deichman's and Le Blanck's method [13].

2.3. Nociception tests

The hot plate test was derived from that of Eddy and Leimbach [14]. The temperature of the plate was maintained at 52 ± 0.4 °C. The tail flick test of D'Amour and Smith [15] modified for mice was used. In both tests, the reaction time was measured 60, 90 and 120 min after administration of the analgetic drugs. Each group consisted of 7–14 mice.

2.4. Statistical analysis

The distribution normality was checked by means of the Kolmogorow-Smirnow test with Lilieforse correction and then variance equality was tested by Fisher's test. The statistical evaluation was performed by means of the Student's t test.

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