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ANTILEUKAEMIC PROPERTIES OF 12-HYDROXYDAPHNETOXIN DERIVATIVES

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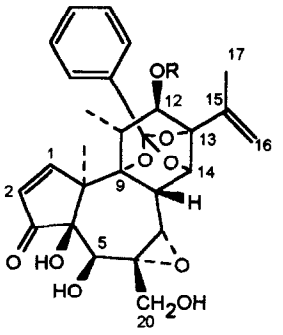
Abstract: A number of 12-acyl derivatives of 12-hydroxydaphnetoxin, 15,16-dihydro-12-hydroxydaphnetoxin, 1,2,15,16,-tetrahydro-12-hydroxydaphnetoxin and their 5,20 acetonides were prepared and their activities determined against murine P388 lymphocytic leukaemia The saturated esters show reduced activity compared with the natural products **2-5**.

The daphnane diterpenoids comprises around thirty natural products isolated from the plant families *Thymelaeaceae* and *Euphorbiaceae*. Most of these compounds possess potent biological properties which include nematicidal, piscicidal and antineoplastic activities.[1] About half of the biologically active compounds belong to the 12-hydroxydaphnetoxin group, typified by the plant toxin mezerein **2**, and are characterised by the presence of 9,13,14-orthoester and 12-unsaturated alkyl ester functionalities. Of these naturally occurring materials nine compounds[2] have been shown to possess significant antineoplastic activity *in vivo* against P388 lymphocytic leukaemia in the mouse. However, the nature of the structural features responsible for the specific antineoplastic activity of this series of compounds is unclear and, in contrast to other classes of naturally occuring antitumour compounds, no systematic study of their activity has yet been carried out.

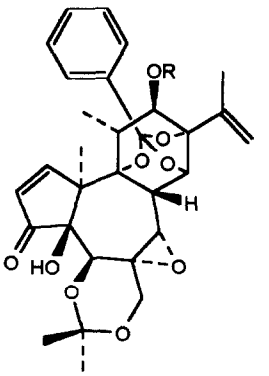
In this preliminary study we have focused on examining the effect of changing the nature of the 12-acyl group of the molecule and have compared the antileukaemic activity of a series of derivatives with the natural products (**2** - **5**) which differ only in the nature of the unsaturated ester group at this position. As a result we show that while an 12-acyl function is necessary for antileukaemic activity, an α,β-unsaturated ester function is not obligatory

Structural modifications

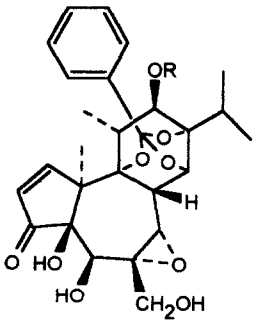
The natural products mezerein **2**, gnidicin **3**, gnididin **4** and gniditrin **5** were isolated from seeds of *Daphne mezerium* and *D. lamprantha* leaf tissue as described previously.[2a,2b] 12-Hydroxydaphnetoxin **1**, $[\alpha]_D$ +46.2°, was prepared in *ca* 90% yield by methanolysis (0.1M NaOMe, 20°, 6h) of **2**.



1 R = H (12-hydroxydaphnetoxin)
2 R = $CO(CH=CH)_2Ph$ (mezerein)
3 R = COCH=CHPh
4 R = $CO(CH=CH)_2 (CH_2)_4 CH_3$
5 R = $CO(CH=CH)_3 (CH_2)_2 CH_3$
6 R = $CO(CH_2)_8 CH_3$
7 R = $COCH_2$(nPrS)CHCH=CH.Ph
8 R = COPh



1a R = H
2a R = $CO(CH=CH)_2$ Ph
3a R = COCH=CHPh
4a R = $CO(CH=CH)_2 (CH_2)_4 CH_3$
5a R = $CO(CH=CH)_3 (CH_2)_2 CH_3$
6a R = $CO(CH_2)_8 CH_3$
8a R = CO Ph



2b R = $CO(CH_2)_4$ Ph
3b R = $CO(CH_2)_2$ Ph
6b R = $CO(CH_2)_8 CH_3$
8b R = CO Ph

2c R = $CO(CH_2)_4$Ph; 1,2-dihydro-
3c R = $CO(CH_2)_2$ Ph, 1,2-dihydro-
6c R = $CO(CH_2)_8 CH_3$, 1,2-dihydro-

The thioether adduct of mezerein **7** was prepared by reaction of **2** with propane thiol in MeOH-phosphate buffer (0.5M, pH 7.2) for 24h Acetonides of compounds **1** -**5** (**1a** -**5a** respectively) were prepared by acid catalysed (4-toluenesulphonic acid, 1mg/cm^3) reaction of the appropriate compounds in acetone Preparation of 12-acyl derivatives of 12-hydroxydaphnetoxin-5,20-acetonide (compounds **6a** and **8a**) was accomplished by addition of a solution of **1a** in CH_2Cl_2-pyridine (4/1, 0 5 cm^3) to solutions of the corresponding acid chloride (5eq) in CH_2Cl_2-pyridine (1/4, 0 5cm^3) and the reactions were allowed to proceed for 24h at 20° before work up. Hydrolysis of acetonides to the corresponding 12-acyl esters (**6** and **8**) was accomplished at room temperature in 20% HCl-MeOH Progressive hydrogenation of the natural products to afford the compounds **2b**, **3b** and **6b** and compounds **2c**, **3c** and **6c** typically involved the following conditions· (a) side chain and $\Delta^{15,16}$ hydrogenation - H_2, 5% Pd/C, EtOAc; (b) side chain, $\Delta^{15,16}$ and $\Delta^{1,2}$ double bond reduction - H_2, 10% Pd/C, EtOH. **8b** was similarly prepared by hydrogenation of **8**. All products were purified by column and thin layer chromatography on SiO_2 and gave satisfactory elemental analyses (or exact mass measurements) and spectroscopic data concordant with their structures.

Structure-Activity Relationships

The effective and toxic doses for *in vivo* activity against murine P-388 lymphocytic leukaemia for the natural products **2** - **5** and some of the derivatives prepared in this work are listed in the table. The natural products **2** - **5**, each of which possess an unsaturated 12-acyl functionality, have similarly narrow dosage ranges for

antileukaemic activity which maximise in the 100-200μg/kg range. It is notable that the parent compound 12-hydroxydaphnetoxin **1** shows no significant tumour inhibitory activity or toxicity even at high dosages indicating that a 12-acyl function is necessary for activity Moreover, the fact that all of the active natural products possess an unsaturated ester function at this position suggests a possible role for this group in alkylation of a biological target molecule *in vivo*.[4] Indeed mezerein **2** is readily alkylated by propane thiol under mild conditions (20°C, pH 7.2) to afford the thioether **7**, a reactivity which might model a biological alkylation. However the product still shows significant, if reduced, tumour inhibitory activity indicating that the presence of a C-12 unsaturated ester function is not necessarily required for activity. It is noteworthy that 12-benzoyldaphnetoxin **8** has similar activity to the propane thiol adduct **7**. With 12-decanoyldaphnetoxin **6**, which lacks any electron rich groups in the side chain, we observe a further reduction of activity.

Table: *In vivo* activity [Expressed in terms of % increase in lifespan (ILS)] of 12-hydroxydaphnetoxin derivatives against murine P388 lymphocytic leukaemia*

Compound	Dosage (mg/kg)				
	0.8	0.4	0.2	0.1	0.05
2	T	T	67	33	33
3	T	T	73	60	N
4	T	T	42	33	33
5	T	T	T	68	44
6	43	42	N	N	N
7	T	35	34	N	N
8	T	49	40	32	N
2b	T	43	43	N	N
3b	T	40	38	N	N
6b	27	30	N	N	N
8b	T	48	42	30	N
2c	27	29	N	N	N
3c	30	25	N	N	N
6c	25	25	N	N	N

* All compounds were administered as suspensions in tween 80 / saline by ip injection on days 1 to 9 following implantation of tumour tissue.[3] The median control survival time was 14 days. Under the same regimen 5-fluorouracil had an ILS value of 65 at 20mg/kg. N = ILS <20%; T = toxic dose; lifespan <85% of control group.

Reduction of the double bonds of the ester groups of **2** and **3** (accompanied by $\Delta^{15,16}$ saturation) affords **2b** and **3b** which possess similar antileukaemic activity to **7** and **8**. This suggests that the 15,16 double bond is of little importance for *in vivo* activity a fact which is reinforced by the similar activities of **8** and **8b**. Reduction of the double bond of the cyclopentenone function to a cyclopentanone as exemplified in **2c** and **3c** results in a further

reduction in activity All of the 5,20-acetonides prepared in the study proved to be inactive over a dosage range of 0.05 to 1 6 mg/kg (data not shown)

On the basis of these results it is clear that the presence of an 12-acyl functionality is a prerequisite for *in vivo* antileukaemic activity in this series of compounds and that the activity is enhanced when this group is unsaturated However it seems unlikely that the mechanism of action simply involves alkylation of a nucleophilic site in a target molecule by this function alone since compounds such as 7 and **8** which lack an α,β-unsaturated ester function still exhibit activity The lower activity of the 12-decanoyl derivative **6** suggests that the presence of an electron rich group in the side chain could be important While the reduction of the $\Delta^{15,16}$ double bond appears to have little effect, the reduction of the $\Delta^{1,2}$ double bond, itself a possible alkylating functionality, further reduces activity The lack of activity of all the 5,20-acetonides could suggest that either or both of the hydroxyl groups are necessary for activity but this must be regarded with caution since these non-polar derivatives may differ dramatically in bioavailability.

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