Synthesis of Calenduladiol Derivatives of Biological Interest

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Abstract

Five calenduladiol derivatives have been synthesized using calenduladiol (**1**) as starting material. Compounds **2** (30-oxo-calenduladiol) and **3** (30-hydroxy-calenduladiol) were obtained by oxidation with SeO_2 while compounds **4** (calenduladiol diacetate), **5** (30-oxo-calenduladiol diacetate) and **6** (30-hydroxy-calenduladiol triacetate) were obtained by esterification with Ac_2O and pyridine from compounds **1**, **2** and **3** respectively. Derivatives **2**-**6** were obtained in moderate - good yields and characterized by ^{1}H and ^{13}C NMR spectroscopy.

Compounds **1**-**6** were screened for acetylcholinesterase (AChE) inhibition using the Ellman's assay. The oxidized analogs **2** and **3** resulted to be the more active ones with 43 % and 40 % of AChE inhibition at 0.2 mM, respectively. Acetylated derivatives (**4**-**6**) elicited a weak activity compared to **1**, at the same concentration.

Keywords

Acethylcholinesterase activity, *Chuquiraga erinacea*, Asteraceae, calenduladiol analogues, pentacyclic triterpenes.

Introduction

Over the last decades triterpenoids have drawn attention because of their anti-inflammatory[1], anti-oedematous[2], antitumor[3], and anti-HIV activities[4]. Abundant in many plants, these metabolites are valuable natural raw materials to perform chemicals modifications and obtain semisynthetic analogs for structure-activity relationship studies.

Asteraceae family constitutes a rich source of several pentacyclic mono-alcohols, diols and triols terpenes such as calenduladiol, faradiol, heliantriol B2, taraxasterol, arnidiol, lupeol, etc.

Acetylcholine serves as a neurotransmitter in the central and peripheral nervous system. The enzyme AChE stops the function of acetylcholine by its hydrolytic destruction in the cholinergic synapses. Enhancement of acetylcholine level in the brain inhibiting AChE is considered one of the most promising approaches for treating Alzheimer's disease.

In the course of our ongoing search of natural AChE inhibitors, we isolated calenduladiol (**1**), in good yields, from ethanolic extract of *Chuquiraga erinacea* D. don. subsp. *erinacea* (Asteraceae). This triterpene elicited moderate AChE inhibition[5] which encouraged us to perform modifications of the chemical groups or the introduction of new funcionalities of the natural triterpene, being obtained five calenduladiol derivatives: 30-oxo-calenduladiol (**2**), 30-hydroxy-calenduladiol (**3**), calenduladiol diacetate (**4**), 30-oxo-calenduladiol diacetate (**5**) and 30-hydroxy-calenduladiol triacetate (**6**), which were evaluated as AChE inhibitors.

Experimental Section

Isolation of Calenduladiol (**1**)

Chuquiraga erinacea D. don. subsp. *erinacea* (Asteraceae) was collected in August 2010 in southwest of Buenos Aires province, Argentina. Dried leaves (170 g) of *C. erinacea* were extracted at reflux with EtOH (2 x 1.5 L, 12 h each) yielding 21.5 g of extract which was partitioned between H_2O/MeOH 50:50 (200 mL) and hexane (150 mL) affording 6.3 g of the crude hexane extract. Column chromatography (silicagel 60, 70-230 mesh) of 4 g of this extract gave 140 mg of calenduladiol (**1**) eluted with hexane/AcOEt 85:15. ^{1}H and ^{13}C NMR and comparison with bibliographic data, permitted the identification of calenduladiol.

Synthesis of calenduladiol derivatives **2-6** has been performed following the scheme of Figure 1.

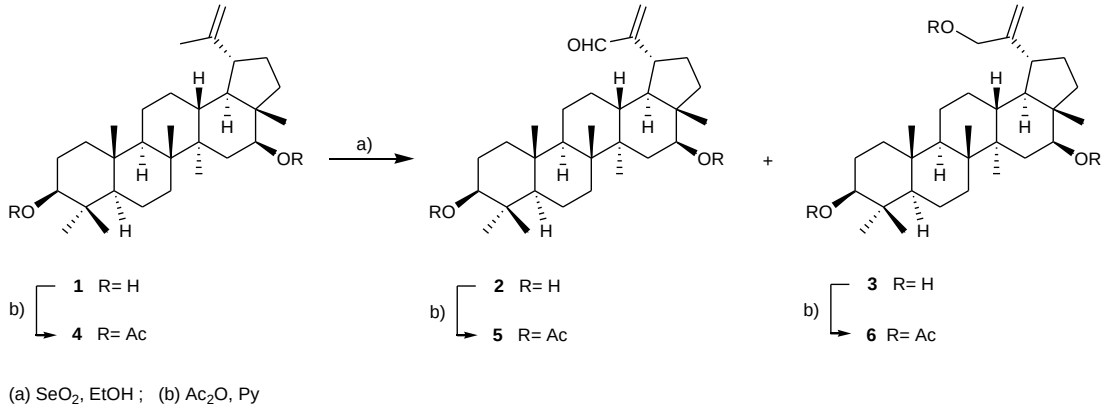


Figure 1

*Synthesis of **2** and **3***

100 mg (0.23 mmol) of calenduladiol in 20 mL of EtOH were treated with 13 mg (0.5 eq) of SeO_2 and the solution was heated under reflux for 20 h. Then the reaction mixture was cooled and EtOH was evaporated *in vacuo*. The crude was treated with water and extracted three times with CH_2Cl_2. The organic layer was dried and concentrated under reduced pressure. The TLC of the crude product showed a mixture of two compounds which were purified by column chromatography (silica gel 60, 230-440 mesh) to obtain **2** (24.9 mg, 0.05 mmol, 24%) and **3** (18.3 mg, 0.04 mmol, 17%) in the fractions eluted with 80:20 and 70:30 hexane- AcOEt, respectively.

*Synthesis **4**, **5** and **6***

Acetic anhydride (1.2 mL) was added to a solution of calenduladiol (20 mg, 0.046 mmol) in 0.6 mL of pyridine, and the mixture was stirred al room temperature for 24 h. The mixture was then poured onto ice and 2 N HCl solution (5 mL), and extracted with CH_2Cl_2 (3 x 6 mL). The combined organic layer was washed successively with water, saturated $NaHCO_3$ solution, and water, dried over anhydrous $MgSO_4$, and evaporated to dryness. The residue was purified by column chromatography on silica gel using CH_2Cl_2 as eluent to give 17.6 mg (0.033 mmol, 72 %) of **4** as a white solid.

The same method was applied for the acetylation of **2** to obtain compound **5** (70% yield), and to the triol **3** to obtain **6** (72 % yield).

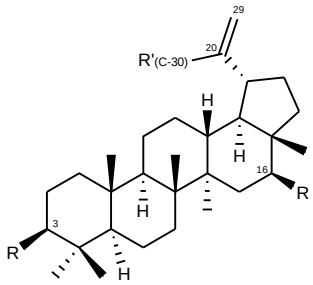
Results and Discussion

Compounds **2** and **3** were obtained with moderate yields by oxidation with SeO_2 in one step. It is to note that derivative **3**, had previously been synthesized in two steps: from oxidation of **1** to the α,β-unsatured aldehyde **2**, and then reduced to **3,** with lower total yields as reported Neukirch *et al*[6],

The derivatives **4**, **5** and **6** were synthesized by esterification with Ac_2O and pyridine from compounds **1**, **2** and **3** respectively and were obtained with goods yields. Compounds **5** and **6** were not found in the literature.

Calenduladiol (**1)** and derivatives **2-6** were characterized by ^{1}H and ^{13}C NMR spectroscopy. In the Table 1 is shown the chemical shifts of C-3, C-16, C-20, C-29, C-30 and acetyl group of these compounds.

Table 1. Chemical shifts of relevant carbons calenduladiol (**1**) and their derivatives **2-6**.



1 R = OH, R' (C-30) = CH_3

2 R = OH, R' (C-30) = COH

3 R = OH, R' (C-30) = CH_2OH

4 R = OAc, R' (C-30) = CH_3

5 R = OAc, R' (C-30) = COH

6 R = OAc, R' (C-30) = CH_2OAc

	δ_c (ppm)[a]					
	1	**2**	**3**	**4**	**5**	**6**
C-3	78.8	78.9	78.9	80.9	80.8	80.8
C-16	76.9	76.9	77.2	79.1	78.8	78.9
C-20	149.9	156.4	154.0	149.8	156.4	148.4
C-29	109.7	133.2	107.4	109.9	130.9	110.8
C-30	19.3	194.8	65.2	19.3	194.9	68.2
COCH_3	-	-	-	170.6, 170.9	170.7, 171.1	170.7, 170.8,
$COCH_3$	-	-	-	21.2, 21.3	21.3, 21.4	21.1, 21.3, 21.4 21.4

[a] Recorded at 75 MHz in CCl_3D.

Compounds **1-6** were screened for AChE inhibition using the Ellman's assay[7]. Enzyme activity was calculated by comparing reaction rates for the samples to the blank as previously reported[8]. All the determinations were performed in triplicate.

The oxidized analogs, **2** and **3** resulted to be the more active ones with a 43 % and 40 % of AChE inhibition at 0.2 mM, respectively. Acetylated derivatives (**4-6**) elicited a weak activity compared to **1,** at the same concentration.

Conclusions

Our results indicate that calenduladiol analogues oxidized at C-30 could be a promising strategy for the enhancement of pharmacological properties of this type of triterpene alcohols.

Acknowledgements

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