A NEOCLERODANE DITERPENOID FROM *SCUTELLARIA GUATEMALENSIS* (LABIATAE)

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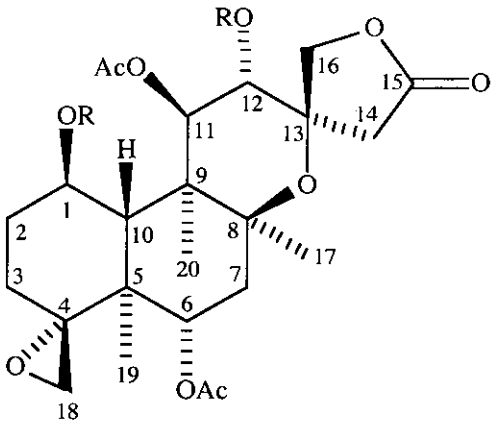
Abstract- A new neoclerodane diterpenoid, (13*R*)-6α,11β-diacetoxy-1β,12α-di*iso*-butyryloxy-4α,18;8β,13-diepoxy-*neo*-clerodan-15,16-olide (Scuteguatemalin) has been isolated from the aerial parts of *Scutellaria guatemalensis* besides the known skullcapflavone II. The structure of the new diterpenoid was established by spectroscopic methods and confirmed by X-Ray analysis.

Scutellaria L., with 360 currently recognized species throughout the World, is one of the largest genera of the Labiatae family.[1] Recently, plants belonging to this genus have attracted an attention due to the interesting biological activities found for some neoclerodane diterpenoids isolated from them, in particular as insects antifeedant[2-4] and against plant pathogenic fungi.[5] In Mexico, *Scutellaria* is represented by 32 species, most of them growing in the mountains nearby the center of the country.[6] As a part of our ongoing chemical studies on Mexican *Scutellaria* spp.,[7] in this paper we report the structure of a new neoclerodane diterpenoid, named scuteguatemalin (**1**), isolated from the aerial parts of *Scutellaria guatemalensis* Leonard (Subgenus Scutellaria, Section Scutellaria)[8] a species used in some parts of the country as a medicinal plant against psychosomatic illness, and some gastrointestinal disorders.[9] The structure of **1** was established from their spectroscopic data and confirmed by X-Ray analysis.

Extraction of the aerial parts of *Scutellaria guatemalensis* afforded, after extensive chromatography, skullcapflavone II[10] and a new neoclerodane diterpenoid to which we have assigned structure (**1**) on the following considerations.

Compound (**1**) was assigned the molecular formula $C_{32}H_{46}O_{12}$ by FAB$^+$, HRMS. Its IR spectrum showed absorptions for a γ-spiro lactone (1789 cm^{-1}) and ester carbonyls (1735 and 1720 cm^{-1}). The ^{1}H and ^{13}C NMR spectra of **1** (Tables 1 and 2) showed signals for two acetates (δ_H 2.03 *s*, 1.98 *s* 3H each; δ_C 170.7 *s*, 169.7 *s*, 21.1 *q* and 20.4 *q*) and two *iso*-butyryloxy groups (δ_H 2.71 *sept*, 2.43 *sept*, 1H each, 1.18 *d* , 1.072 *d* , 1.071 *d* and 1.05 *d* , 3H each; δ_C 176.7 *s*, 175.6 *s*, 33.8 *d*, 33.6 *d*, 19.9 *q*, 18.7 *q*, 18.4 *q* and 17.7 *q*).[11] In addition these spectra showed the characteristics signals for the methyl groups of a neoclerodane diterpene[12] (δ 1.39 *s* Me-17, 24.0 *q* C-17; 1.38 *s* Me-19, 15.7 *q* C-19; 1.09 *s* Me-20, 18.3 *q* C-20).

Two one-proton doublets (AB system) at δ 3.18 (J = 4 Hz) and δ 2.51 were assigned to the protons of a 4α,18-oxirane and two additional AB systems at δ 3.03, 2.65 (1H each, *d*, J= 16.8, H-14 pro-*R* and H-14 pro-*S* respectively) and 4.45, 4.22 (1H each, *d*, J=9.5 Hz, H-16 pro-*S* and H-16 pro-*R* respectively) were attributed to the protons of a γ-spiro lactone.[13,14] Other relevant signals, in the ^{1}H NMR spectrum of **1**, are those due to the four geminal protons of the ester groups at δ 5.59 (*dt*, J=3.8, 6.5), 4.97 (*dd*, J= 5.3, 11.3), 5.54 (A part of an AB system *d*, J=11.5) and 5.63 (B part). A doublet at δ 2.57 (J=3.8 Hz) was attributed to H-10. These functional groups have been frequently found in other neoclerodane derivatives previously isolated from *Scutellaria* species.[13-15]



1, R =-COisoBut

Table 1.^{1}H NMR Data for Scuteguatemalin (**1**)†

H	δ	J	H	δ	J
1	5.59 dt	3.8, 6.5	16 pro *R*¥	4.22 d	9.5
2 α	2.13 m		3H-17	1.39 s	-
2 β	1.94 m		18 pro *Z*¥	3.18 d	4
3 α	2.03 m		18 pro *E*¥	2.51 d	4
3 β	1.61 ddd	8.3, 10.8, 19	3H-19	1.38 s	-
6	4.97 dd	5.3, 11.3	3H-20	1.09 s	-
7 α	1.82 dd	11.3, 14	$(CH_3)_2CHCOO$	2.71 sept	7
7 β	1.76 dd	5.3, 14		2.43 sept	7
10	2.57 d	3.8	$(CH_3)_2CHCOO$	1.18 d	7
11	5.54 d	11.5		1.072 d	7
12	5.63 d	11.5		1.071 d	7
14 pro *R*¥	3.03 d	16.8		1.05 d	7
14 pro *S*¥	2.65 d	16.8	CH_3CO	2.03 s	-
16 pro *S*¥	4.45 d	9.5		1.98 s	-

†Run at 500 MHz. $CDCl_3$, TMS, J in Hz. ¥Distinguished by NOESY spectrum.

The location of the ester substituents in compound (**1**) was established from the heteronuclear multiple bond connectivity (HMBC) and the heteronuclear multiple quantum coherence (HMQC) spectra. The HMBC showed cross peak of correlation through three bonds between the signal at δ175.6 (*iso*-butyryloxy carbonyl) with the proton at δ 5.63 (B part of an AB system, *d*, J = 11.5, H-12). This proton correlated in the HMQC spectrum with the signal attributed to C-12 (δ 70.8), which in his turn showed cross peaks in the HMBC spectrum with both protons of the lactone group (H-16 pro-*R* and H-16 pro-*S*). These facts establi-

shed one of the *iso*-butyryloxy groups at the C-12 position. On the other hand, the signal at δ 170.7 (acetate carbonyl) correlated in the HMBC spectrum with the proton at δ 5.54 (A part of an AB system, J = 11.5, H-11). In complete agreement with these facts, the signal attributed to C-11 (δ 73.0, *d*) correlated through three bonds with the Me-20 protons and H-10 (2.57, *d*, J=3.8), thus confirming the location of one of the acetate groups at the C-11 position. The coupling constants between H-11 and H-12 indicated an antiperiplanar relation between these protons and therefore an *equatorial* orientation for both ester moieties as depicted in **1**. The signal, in the ^{13}C NMR spectrum of **1**, at δ 169.7 (acetate carbonyl) showed cross peaks of correlation in the HMBC spectrum with the double doublet at δ 4.97 (H-6, J = 5.3, 11.3) indicating that the other acetate group is located at the C-6 position with an *equatorial* orientation. The remaining *iso*-butyryloxy group must be located therefore at C-1, according to the multiplicity observed for the geminal proton of this ester (δ 5.59, *dt*, H-1, J =3.8, 6.5 Hz) and the HMBC correlations observed for C-1 (with H-10 and H-2β).

The relative configuration depicted in **1** was firmly established from its NOESY spectrum (Figure 1). The H-14 pro-*S* showed a strong NOE cross peak with Me-17 indicating a 13*R* stereochemistry. On the other hand, the H-14 pro-*R* correlated with the signal ascribed to H-11, confirming in this way the orientation proposed for the acetate group at C-11. While H-6β axial correlated only with H-10 and H-18 pro-*Z*, the H-7α *axial* showed cross peaks with the Me-19 and Me-20. These facts besides the cross peak of Me-20 protons with H-1, Me-17 and Me-19 protons, indicated that H-7α *axial*, Me-20, Me-17, Me-19 and H-1 are on the same side of the decaline and H-10 and H-6β *axial* are on the opposite one. These results established a *cis* junction between B and C rings, in agreement with the NOE correlation between H-16 pro-*S* with H-12β. Other relevant cross peaks in the NOESY spectrum of **1** are those between H-1 with H-10, H-2α and H-2β. In his turn H-2α correlated with Me-19. The chemical shifts observed, in the $^{13}CNMR$ spectrum

Table 2. ^{13}C NMR Data for Scuteguatemalin (**1**)¶

C	δ	C	δ	C	δ
1	70.4 *d*	13	79.7 *s*	O<u>C</u>OCH(CH$_3$)$_2$	176.7 *s*
2	25.6 t	14	38.2 t		175.6 s
3	26.4 t	15	173.5 s	OCO<u>C</u>H(CH$_3$)$_2$	33.8 d
4	63.8 s	16	77.8 t		33.6 d
5	40.3 s	17	24.0 q	OCOCH(<u>C</u>H$_3$)$_2$	19.9 q
6	69.0 d	18	54.1 t		18.7 q
7	38.6 t	19	15.7 q		18.4 q
8	82.0 s	20	18.3 q		17.7 q
9	46.1 s	O<u>C</u>OCH$_3$	170.7 s		
10	46.8 d		169.7 s		
11	73.0 d	OCO<u>C</u>H$_3$	21.1 q		
12	70.8 d		20.4 q		

¶Run at 125 MHz. $CDCl_3$, TMS. Assignments confirmed with the aid of HMBC and HMQC spectra.

of **1**, for the Me-19 and Me-20 (Table 2) indicated a *trans* fusion for the A/B rings.[16] The coupling constants found for H-10 and H-1 (J=3.8 Hz, Table 1) and the NOE correlation between H-10 with H-1 and Me-19 protons with H-2α, led us to establish that the A ring is in a boat conformation, due to the presence of steric effects between the isobutyryloxy moiety at C-1 with the ester groups at C-11 and C-12 (Dreiding Models). The structure of **1** was confirmed by X-Ray diffraction analysis of a single crystal. The molecular structure is illustrated in Figure 2. This analysis confirmed the 13*R* configuration and the location of the ester groups. Although the absolute configuration of **1** was not ascertained, we can assume, on biogenetic grounds, that **1** belongs to the neoclerodane series as other diterpenoids isolated from *Scutellaria* spp whose absolute configuration was firmly established by X- Ray diffraction analysis[4,7,17] or the CD exciton chirality method.[18,19]

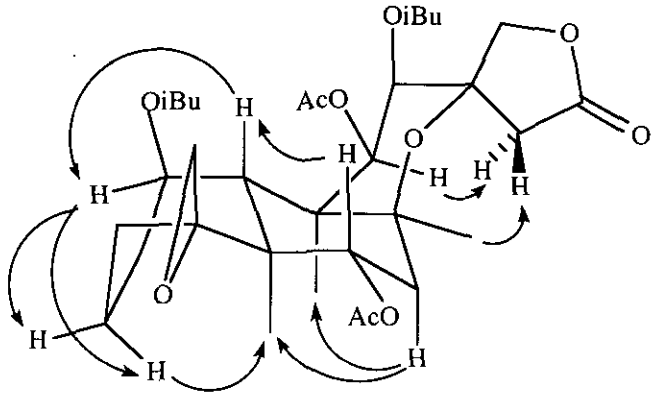


Figure 1. NOESY correlations of **1**

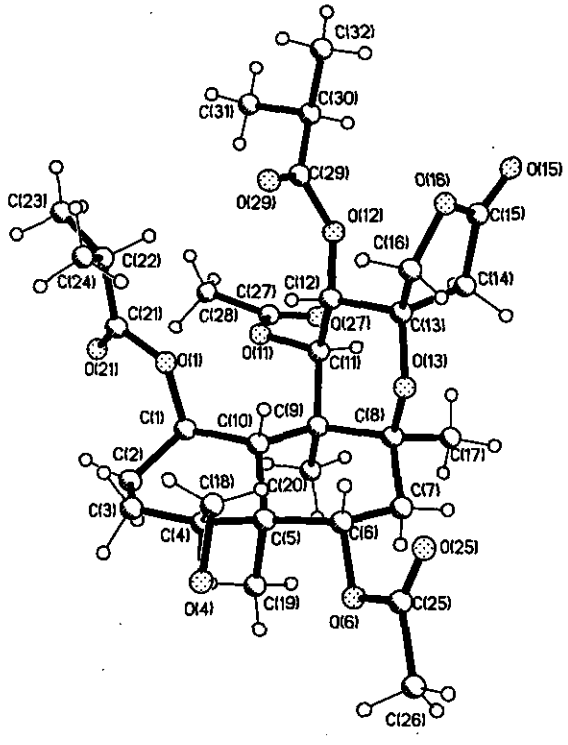


Figure 2. Computer-generated perspective drawing of scuteguatemalin (**1**).

From a chemotaxonomic point of view, it is of interest to note that scuteguatemalin (**1**) lacks an oxygenated substituent at C-19 commonly found in several neoclerodane diterpenoids from European *Scutellaria* species.[15] Although compound (**1**) shares this feature with scutebaicalin from *S. baicalensis*[19] and with the neoclerodane diterpenoids isolated from the Mexican species *S. drummondii*,[7] *S. seleriana*,[22] *S. caerulea* and the Chinese plant *S. rivularis*,[20,21] the oxidation at C-12 is a unique feature of **1**. Although the oxidation at C-12 is frequently found in neoclerodane diterpenoids of other genera of the Labiatae family such as *Teucrium* and *Salvia*,[23] to the best of our knowledge, this is the first example of a neoclerodane diterpenoid from *Scutellaria* plants with oxidation at C-12.

EXPERIMENTAL

Mps are uncorrected. *Scutellaria guatemalensis* was collected in the State of Puebla (México) in October 1995. Voucher specimens (MEXU MA-2876) (IMSSM MA-2903) were deposited at the herbaria of the Instituto de Biología UNAM and Instituto Mexicano del Seguro Social respectively.

Extraction, fractionation and isolation of Scuteguatemalin from Scutellaria guatemalensis. Dried and powdered aerial parts of *S. guatemalensis* (189.5 g) were extracted x 2 with Me_2CO (4 L) for 8 days at rt . The solvent of the combined extracts was removed in *vacuo* to yield 4.6 g of a gummy residue which was subjected to vacuum chromatography over silica gel. Mixtures of petrol-Me_2CO of increasing polarity were used as eluents. From the first fractions eluted with petrol-Me_2CO (4:1) scuteguatemalin (**1**) (11.1 mg) was isolated after crystallization with MeOH. Flash chromatographic (C_6H_6-Me_2CO; 24:1) purification of some other fractions eluted with the same polarity afforded an additional crop of **1** (24.4 mg). Skullcapflavone (3.5 mg) was isolated from the last fractions eluted with petrol-Me_2CO (8:2), the physical data obtained (mp, MS, IR and ^1H NMR) were identical with those published in the literature.[10]

Scuteguatemalin (**1**). Crystalline solid, mp 233-234°; $[\alpha]_D$ -108° (c 0.1; MeOH); IR ν_{max} ($CHCl_3$) cm^{-1}: 1789, 1735, 1720, 1469, 1373 1242, 1193, 1153, 1140, 1043; ^1H NMR see Table 1; ^{13}C NMR see Table 2; FAB$^+$ MS m/z (rel. int.): 645 (M^++ Na), 623 (M^++ 1), 535 (5), 493 (3), 475 (35), 465 (30), 457 (5), 415 (5), 327 (15), 203 (15), 185 (15), 137 (25), 119 (15), 71 (75) 43 (100).$C_{32}H_{46}O_{12}$ requires M^+ at m/z 622.3068 found HRMS 622.3071.

X-Ray structure determination of scuteguatemalin (**1**).The colorless crystal of **1**, were obtained by slow evaporation from MeOH. The data were collected on a Siemens P3/F diffractometer. Intensities were collected at rt using nickel filtered Cu Kα radiation (λ = 1.54178 Å) , and were corrected for background, Lorentz and polarization effects. The structure was solved by direct methods and refined by full-matrix least-squares[24] with anisotropic temperature factor for the non-hydrogen atoms. The hydrogen atoms were included at idealized positions, all hydrogens with a fixed temperature factor U = 0.08 $Å^2$.

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