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Helivypolide G. A novel dimeric bioactive sesquiterpene lactone

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Abstract—In the course of our ongoing research for new allelochemicals from *Helianthus annuus*, a novel dimeric bioactive sesquiterpene lactone, helivypolide G has been isolated and characterized from the medium polar active fractions of the leaves of cultivar variety Stella. The monomers are connected through carbons C-15 of each unit and an oxygen bridge, forming an enolic oxane ring.

One of the most abundant groups of secondary metabolites in the *Helianthus* (Compositae) genus are sesquiterpenes (especially guaianolides and heliangolides).[1] In the course of our ongoing research for new allelochemicals from *Helianthus annuus*, here we report the isolation and characterization of a novel dimeric sesquiterpene lactone from the medium polar active fractions of the cultivar variety Stella.

Dimeric sesquiterpene lactones other than those that are guaianolide-derived[2] are rare compounds. However, some dimeric eudesmanolides,[3] xantanolide[4] and germacranolide[5] have been reported. Also, the connection of monomers through carbons C-15 has been reported once[6] and there is only one structure reported with a spirocyclic dihydropyrane ring connecting the two sesquiterpene lactone monomers.[7] Finally, the bridging ether oxygen from C-5 to C-10 in a germacranolide skeleton is also unusual.[8] In this communication we report a novel bioactive sesquiterpenoid dimer named helivypolide G (**1**) that presents all of these uncommon characteristics. It was isolated from the leaves of *H. annuus* cv. Stella. Moreover, **1** inhibits wheat coleoptile growth[9] 72% and 50% at 1 and 0.1 mM ($P < 0.01$), respectively.

Fresh leaves of *H. annuus* cv. Stella were extracted in water (1/3, w/v) for 24 h at room temperature in the dark. The aqueous solution was extracted with CH_2Cl_2 at room temperature, and the solvent of the organic layer was removed by reduced pressure evaporation. The resulting crude extract was chromatographed on a silica gel column (eluted with hexane/EtOAc, from 100:1 to 1:1) and further purified by HPLC using hexane/acetone (4:1) as eluent on a silica gel column to give helivypolide G (Fig. 1).

Compound **1** was isolated as a yellowish amorphous solid The IR spectrum presents bands at 1771 cm^{-1} (α,β-unsaturated lactone), 1730 cm^{-1} (α,β-unsaturated ester) and at 1672 cm^{-1} (α,β-unsaturated ketone). The HR-MS spectrum presents the molecular ion at *m*/*z* 716.2801, which corresponds to a molecular formula $C_{40}H_{44}O_{12}$ (calculated 716.2832), as well as fragments at 633 $[M-C_5H_7O]^+$, 358 $[M-C_{20}H_{22}O_6]^+$, and 83 $[C_5H_7O]^+$.

The ^1H NMR spectrum (Table 1) presents duplicated typical signals of a sesquiterpene lactone with a heliangolide backbone: H-5 (δ 4.42 and 4.05); H-6 (δ 4.84 and 5.19); H-7 (δ 3.47 and 3.33) and the exocyclic methylene moiety H-13a, H-13″a (δ 5.56 and 5.58) and H-13b, H-13″b (δ 6.24 and 6.27). These observations together with the previous data indicate that this compound is, in fact, a dimer of a sesquiterpene lactone.

Keywords: Sesquiterpene lactone; Sesquiterpene dimer; Heliangolide; Helivypolide G; *Helianthus annuus* cv. Stella.

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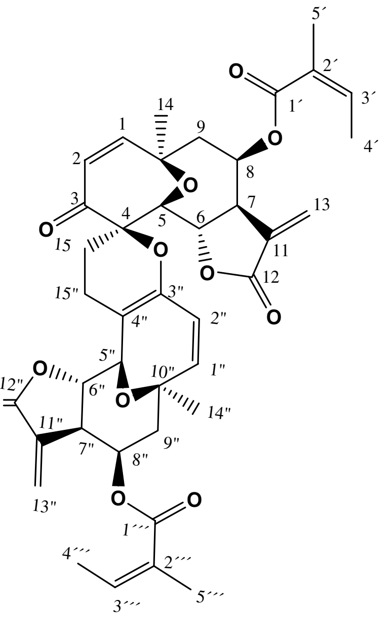

Figure 1. Structure of helivypolide G.

The ^{1}H NMR 2D COSY experiment allows us to correlate two typical doublets from an exocyclic double bond at δ 6.24 (1H, d, $J_{7,13b}=3.5$ Hz, H-13b) and δ 5.56 (1H, dd, $J_{7,13a}=2.9$ Hz, H-13a), with the proton at δ 3.47 (1H, ddd, $J_{6,7}=9.5$, $J_{7,8}=3.2$, $J_{7,13a}=2.9$, $J_{7,13b}=3.5$ Hz) assigned to H-7. It is also observed that the signal of H-7 at δ 3.47 presents coupling with a signal at δ 5.74 (1H, ddd, $J_{7,8\alpha}=3.2$, $J_{8\alpha,9\alpha}=3.2$, $J_{8\alpha,9\beta}=3.9$ Hz) assigned to the H-8α. This is correlated with the signals of the protons H-9β (δ 2.15, 1H, dd, $J_{9\alpha,9\beta}=15.5$, $J_{9\beta,8\alpha}=3.9$ Hz) and H-9α (δ 2.34, 1H, dd, $J_{9a,9b}=15.4$, $J_{9\alpha,8\alpha}=3.2$ Hz). The angelate moiety should be located at C-8 as indicated by the chemical shift of H-8 at 5.75.

On the other hand, the signal at δ 3.47 (H-7) can be correlated with that at δ 4.84 (1H, dd, $J_{5,6}=9.3$, $J_{7,6}=9.5$ Hz) assigned to H-6, which shows coupling with the signal at δ 4.42 ($J=9.3$ Hz) corresponding to H-5. The chemical shift of H-5 suggests the presence of an oxygen at this position and it should be bonded to a quaternary carbon, since H-5 is only coupled with H-6.

The gHMBC spectrum allows us to correlate the proton at δ 4.42 (H-5) with a methylenic carbon at δ 27.2 (C-15) and with two quaternary carbons at δ 197.0 (carbonyl group at C-3) and δ 83.8 (C-4). This carbon correlates in same experiment with the signals at δ 4.84 and 5.95, assigned to H-6 and H-2. On the other hand, in the 2D COSY experiment the signal at δ 5.95 (H-2) has a correlation with a signal at δ 6.29 ($J=13.5$, H-1). These signals appear as an isolated AB system, indicating the presence of a carbonyl group in C-3. A singlet at δ 1.33 agrees with a methyl group attached to a quaternary carbon bearing oxygen atom at C-10. The previous spectroscopic data allow the proposal of the substructure A shown in Figure 2, where an ether bridge links carbons C-5 and C-10.

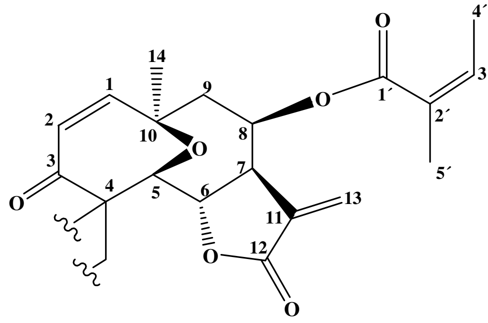

Figure 2. The partial structure A of helivypolide G.

The second set of signals correlate as follows: in the ^{1}H NMR 2D COSY spectrum, the signal at δ 3.33 (1H, ddd, $J_{6,7}=9.7$, $J_{7,8}=3.2$, $J_{7,13a}=3.1$, $J_{7,13b}=3.5$ Hz, H-7″) is connected with two doublet signals typical for an exocyclic methylene at C-13 in a γ-lactone ring at δ 6.27 (1H, d, $J_{7'',13''b}=3.5$ Hz, H-13″b) and δ 5.58 (1H, dd, $J_{7'',13''a}=3.1$ Hz, H-13″a). The coupling between the signal of the proton H-7″ and H-8″α (δ 5.74, 1H, ddd, $J_{7'',8''\alpha}=3.2$, $J_{8''\alpha,9''\alpha}=3.2$, $J_{8''\alpha,9''\beta}=3.9$ Hz) is also observed. The latter having coupling with the protons H-9″β (δ 1.88, 1H, dd, $J_{9''\alpha,9''\beta}=15.5$, $J_{9''\beta,8''\alpha}=3.9$ Hz) and H-9″α (δ 2.34, 1H, dd, $J_{9''\alpha,9''\beta}=15.5$, $J_{9''\alpha,8''\alpha}=2.7$ Hz). The angelate ester should be located at C-8″ based on the chemical shift of the signal of H-8″ at δ 5.74. H-7″ and is also correlated with the signal at δ 4.84 (1H, dd, $J_{5'',6''}=9.5$, $J_{7,6}=9.7$ Hz) of H-6″, which shows coupling with the signal at δ 4.05 ($J=9.5$ Hz) corresponding to the proton at C-5″. The chemical shift of H-5″ indicates the presence of an oxygen at this position; finally C-5″ must be bonded to a quaternary carbon, since it has coupling only with H-6″, as occurs in the substructure A.

In the ^{1}H NMR spectrum it can be observed that the singlet signal at δ 5.88 integrates for 2H. This signal in the gHSQC spectrum is correlated with two carbons at δ 140.6 and 122.4, and can be assigned to the protons at C-1″ and C-2″ that are overlapping. In the two dimensional ^{1}H–^{13}C NMR spectrum to more than three bonds (gHMBC), these signals are correlated with other two signals for quaternary carbon at δ 145.4 (C-3″) and 114.0 (C-4″). The presence of another singlet methyl group at δ 1.32 and the signals corresponding to the second angelate moiety allows us to establish the following partial structure B (Fig. 3).

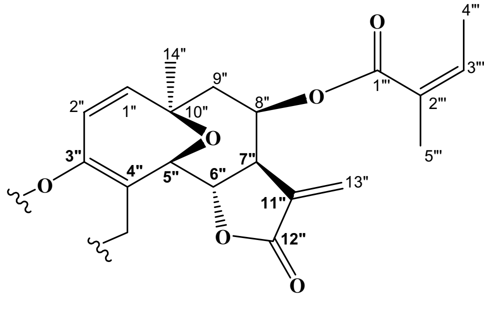

Figure 3. The partial structure B of helivypolide G.

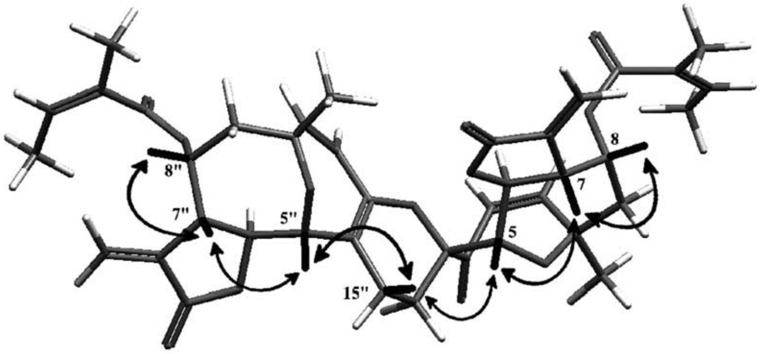

Figure 4. Selected NOE effects of helivypolide G.

Table 1. NMR spectral data of helivypolide G

No	δ_H	δ_C	No	δ_H	δ_C
1	6.29, d (13.5)	150.2d	1″	5.88, s	140.6d
2	5.95, d (13.5)	123.7d	2″	5.88, s	122.4d
3		197.4s	3″		145.4s
4		83.8s	4″		114.0s
5	4.42, d (9.3)	73.4d	5″	4.05, d (9.5)	78.0d
6	4.84, dd (9.3,9.5)	75.9d	6″	5.19, dd (9.5, 9.7)	77.4d
7	3.47, dddd (9.5, 3.2, 2.9, 3.5)	47.3d	7″	3.33, dddd (9.7, 3.2, 3.1, 3.5)	46.8d
8	5.74, ddd (3.2, 3.2, 3.9)	64.9d	8″	5.74, ddd (3.2, 2.7, 3.9)	65.8d
9	α, 2.15, dd (15.5, 3.9)	46.6t	9″	α, 2.21, dd (15.5, 2.7)	46.8t
	β, 2.36, dd (15.5, 3.2)			β, 1.88, dd (15.5, 3.9)	
10		79.9s	10″		79.7s
11		133.7s	11″		134.5s
12		168.8s	12″		168.3s
13	5.56, d (2.9), 6.24, d (3.5)	121.5t	13″	5.58, d (3.1), 6.27, d (3.5)	121.8t
14	1.33, s	31.1q	14″	1.32, s	31.4q
15	1.95, ddd (10.4, 7.6, 3.6)	27.2t	15″	2.30, ddd (11.7, 6.1, 3.6)	22.3t
	2.29, ddd (10.4, 6.1, 4.4)			2.50, ddd (11.7, 7.6, 4.4)	
2′		126.1s	2⁗		126.1s
3′	6.04, qq (7.3, 1.5)	139.9d	3‴	6.04, qq (7.3, 1.5)	139.9d
4′	1.93, dq (7.3, 1.5)	15.6q	4⁗	1.93, dq (7.3, 1.5)	15.6q
5′	1.76, dq (1.5, 1.5)	20.3q	5‴	1.76, dq (1.5, 1.5)	20.3q

600 MHz for ^1H NMR, 125 MHz for ^{13}C NMR and DEPT. Coupling constants in Hz appear in parentheses.
Chemical shifts are given in ppm with respect to residual $CHCl_3$ or $CDCl_3$ signals for ^1H and ^{13}C NMR spectra (δ 7.25 and 77.00, respectively).

The union of these two partial structures can be proposed through a cyclohexene ring with a spiranic centre at C-4. This ring is formed through bonding of the two coupled methylenes, at C-15 and C-15″, as well as the oxygen at C-3″ and the carbon C-4. The ^1H–^{13}C NMR experiment (gHMBC) allows us to correlate the signals at δ_H 4.42, 4.84 and 5.95 corresponding to the protons H-5, H-6 and H-3 with the quaternary carbon C-4, and the signal of H-5 (δ_H 4.42) with the methylene at δ_C 27.2 (C-15). This structure justifies the chemical shift of the quaternary carbon C-4 at δ 83.8 and the absence of a carbonyl group in the partial structure B. The α orientation of the proton at C-5 can be deduced from the NOE's (Fig. 4) with H-7 and H-8. The relative stereochemistry of the spiranic carbon (C-4) is inferred from the positive NOE effect observed between the signals of the protons H-5 and H-15. Other selected NOE effects are showed in Figure 4 and confirm the stereochemistry and the structure proposed. Consequently, the data presented are consistent with the structure described in Figure 1, which has not been previously described in the literature and is named here helivypolide G.

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