

CLIBADIOLIDE, A SESQUITERPENE LACTONE ESTERIFIED WITH A HOMODITERPENE FROM *CLIBADIUM PITTIERII*

G. TAMAYO-CASTILLO, J. JAKUPOVIC, F. BOHLMANN and V. CASTRO*

Institute of Organic Chemistry, Technical University of Berlin, D-1000 Berlin 12, F.R.G.; *Universidad de Costa Rica, Ciudad Universitaria Rodrigo Facio, Costa Rica

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Abstract—The aerial parts of *Clibadium pittieri* afforded in addition to known melampolides two new ones, one being esterified with a homoditerpenic acid and the homoditerpenic acid itself. The structures were elucidated by high field NMR spectroscopy. The chemotaxonomy is discussed briefly.

INTRODUCTION

The investigations of the chemistry of the genus *Clibadium* have led to the isolation of several flavones [1-3] and some acetylenic compounds, especially ichthyotherol derivatives, have been isolated from the roots [4]. Benzfuranes are reported from one species [5]. We now have reinvestigated *C. pittieri* Greenm. from which so far only flavones are reported [3].

RESULTS AND DISCUSSION

The aerial parts of *C. pittieri* afforded *ent*-kaur-16-en-19-oic acid as the main constituent, hex-2*E*-enoic acid, the melampolides **1** [6], **2** [7], **3** [8] and **4**, the homoditerpene **6** and the unusual sesquiterpene lactone **5**, a melampolide esterified with a homoditerpenic acid **6**.

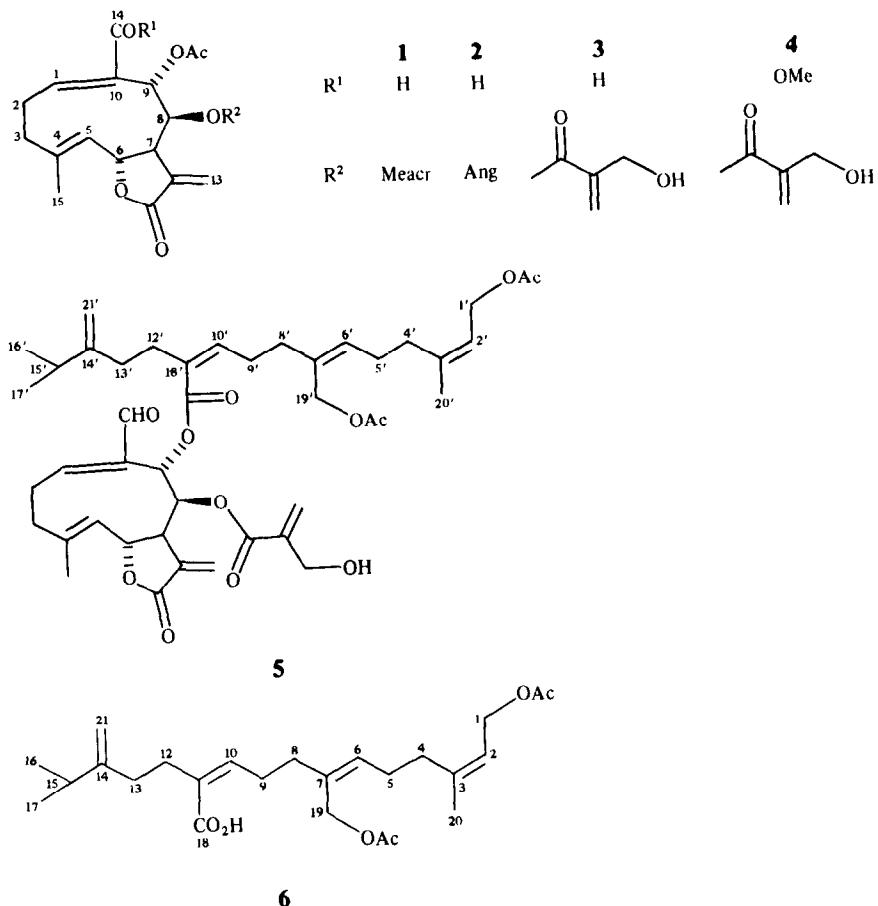
The structures of the lactones **1**, **2** and of the major lactone **3** followed from the ¹H NMR spectra which were identical with those of authentic material. The spectrum of **4** (Table 1) indicated that this lactone was closely related to **3** but the aldehyde group was replaced by a carbomethoxy group. Accordingly, the low field signal of an aldehyde proton was replaced by a methoxy singlet at δ 3.81 and a few signals were slightly shifted. Thus lactone **4** was 19-hydroxy-15-desoxyorientalide.

As in similar cases the acid **6** gave no molecular ion. However, the ¹H NMR spectrum (Table 1) clearly showed that a geranylnerol derivative with an additional methylene carbon was present. This was in agreement with the fragment *m/z* 314 which corresponds to $C_{21}H_{30}O_2$ formed by loss of two molecules of acetic acid. The presence of two acetate groups clearly followed from the ¹H NMR spectrum while in the mass spectrum no elimination of acetic acid from *m/z* 314 was visible. The fragment *m/z* 269 indicated the presence of a carboxyl group by loss of CO_2H from *m/z* 314. Furthermore, a low field triplet at δ 5.99 supported the presence of a conjugated acid group. The relative position of the oxygen functions and the exomethylene group was determined by spin decoupling and NOE difference spectroscopy. Thus,

the H-15 signal could be assigned by irradiation of the six proton doublet (H-16, H-17). As the H-15 signal was sharpened by irradiation of the olefinic methylene signals the exomethylene group was at C-14. Furthermore starting with H-1 (δ 4.55 *br d*), H-2, H-4 and H-20 were assigned. Accordingly, the second acetoxy group was at C-18 or C-19. Due to the carboxyl group the signals of two of the allylic methylene groups were shifted downfield (δ 2.61 *br q* and 2.38 *br t*). Irradiation of the latter sharpened the low field triplet at δ 5.99 and showed a clear NOE with one of the H-21 protons establishing the position of the acid group at C-11 and of the acetoxy group at C-19, respectively. Further NOE's between H-20 and H-2, between H-19 and H-5 as well as between H-10 and H-12, ensured the configurations of the double bonds. Thus the complete structure and stereochemistry of the acid, which we have named clibadic acid, were determined.

The molecular formula of compound **5** could not be determined by high resolution mass spectrometry. The highest peak corresponded to $C_{40}H_{50}O_8$, formed by loss of two molecules of acetic acid as in the case of **6**. The ¹H NMR spectrum (Table 1) showed strong similarities to that of **6** and to that of **3** and only small shift differences for the signals of H-9' and H-10' as well as of H-9 indicated a connection of 9-*O*-desacetyl **3** and **6** at C-9. Accordingly, the compound **5** was the 9-desacetyl derivative of **3** esterified with the acid **6**. The fragmentation pattern in the mass spectrum of **5** also supports the structure. Thus the fragments *m/z* 314 and 313 correspond to the acid part after loss of two molecules of acetic acid while *m/z* 243 probably is due to the lactone part after elimination of the acid and an acyloxy group. As far as we know this is the first example of a sesquiterpene lactone which bears a diterpene or homoditerpene as an ester residue.

The isolation of melampolides and geranylnerol derivatives from a *Clibadium* species is of chemotaxonomic interest as the position of the genus is in question. Previous interpretations placed it in the Melampodiinae [9].



More recently the genus was placed in the Milleriinae [10] and also in the new subtribe Clibadiinae together with *Reincourtia* and *Lantanopsis* [11]. Melampolides are reported from the subtribes Melampodiinae, Milleriinae and Enhydrinae but geranylnerol derivatives oxygenated at C-18 are present only in *Melampodium* [12, 13], *Acanthospermum* [14, 15], *Ichthyothere* [8] and *Smallanthus* [16]. The latter also contains homoditerpenes with a 14-methylene group. The co-occurrence of acetylenes related to ichthyotherol, both in *Clibadium* and *Ichthyothere*, also is of interest. This complex clearly needs further investigations concerning the taxonomy and the chemistry.

EXPERIMENTAL

The air-dried aerial parts (620 g, collected near La Cinchona, Costa Rica, in September 1986, voucher 118842CR, deposited in the Herbarium of the University of Costa Rica) were extracted with MeOH-Et₂O-petrol (1:1:1) at room temp. CC (silica gel) of the defatted extract afforded two crude fractions (Et₂O-petrol, 1:2 and Et₂O-MeOH, 4:1). The less polar fraction gave *ent*-kaurene-16-en-19-oic acid (400 mg). The polar fraction was separated again by flash chromatography (silica gel, ϕ 30-60 μ , Et₂O-petrol mixtures) affording four fractions

(2/1-2/4). TLC (Et₂O-petrol, 4:1) and HPLC (RP 18, MeOH-H₂O, 9:1) of fraction 2/1 gave 2E-hexenoic acid (1 mg) and **6** (2 mg) (*R*, 7.8 min). TLC of fraction 2/2 (Et₂O-petrol, 6:1) gave a mixture which afforded by HPLC (MeOH-H₂O, 17:3) **4** (5 mg) (*R*, 4.9 min.), **1** (7 mg) (*R*, 5.4 min.), **2** (2 mg) (*R*, 5.9 min.) and **5** (6 mg) (*R*, 15.8 min.). TLC of 2/3 (Et₂O-petrol, 4:1, two developments) gave **1** (2 mg) and **4** (16 mg). TLC of 2/4 gave **3** (200 mg). Known compounds were identified by comparing the 400 MHz ¹H NMR spectra with those of authentic material.

19-Hydroxy-15-desoxyorientalide (**4**). Colourless gum; IR $\nu_{max}^{CHCl_3}$ cm⁻¹: 3610 (OH), 1768 (γ -lactone), 1745 (OAc), 1725 (C=CCO₂R); MS *m/z* (rel. int.): 434.158 [M]⁺ (0.4) (calc. for C₂₂H₂₆O₉: 434.158), 374 [M-HOAc]⁺ (3.5), 272 [374-RCO₂H]⁺ (44), 85 [RCO]⁺ (100); $[\alpha]_D$ +54 (CHCl₃; *c* 0.35).

Clibadiolide (**5**). Colourless gum; IR $\nu_{max}^{CHCl_3}$ cm⁻¹: 3490 (OH), 1770 (γ -lactone), 1740 (OAc), 1690 (C=CCO); MS *m/z* (rel. int.): 658.349 [M-2 \times AcOH]⁺ (1.2) (calc. for C₄₀H₅₀O₈: 658.351), 345 [658-RCO₂]⁺ (1.5), 314 (14), 313 (16), 243 (345-RCO₂)⁺ (60), 133 (64), 85 (74), 69 (54), 55 (100).

Clibadic acid (**6**). Colourless gum; MS *m/z* (rel. int.): 314.225 [M-2 \times AcOH]⁺ (3) (calc. for C₂₁H₃₀O₂: 314.225), 271 [314-CHMe₂]⁺ (3), 269 [314-CO₂H]⁺ (5), 133 [C₁₀H₁₃]⁺ (64), 83 (54), 55 (100).

Table 1. ^1H NMR spectral data of compounds **4–6** (400 MHz, CDCl_3 , δ -values)

H	4*	5	H	5†	6†
1	7.02 <i>dd</i>	6.79 <i>dd</i>	1'	4.56 <i>br d</i>	4.55 <i>br d</i>
2 ₁	2.64 <i>dddd</i>	2.88 <i>dddd</i>	2'	5.37 <i>br t</i>	5.39 <i>br t</i>
2 ₂	2.49 <i>m</i>	2.66 <i>m</i>	4'	2.18 <i>m</i>	2.18 <i>m</i>
3 ₁	2.42 <i>ddd</i>	2.52 <i>ddd</i>	5'		
3 ₂	2.05 <i>br dd</i>	2.10 <i>m</i>	6'	5.42 <i>br t</i>	5.41 <i>br t</i>
5	4.98 <i>br d</i>	4.94 <i>br d</i>	8'	2.18 <i>m</i>	2.18 <i>m</i>
6	5.13 <i>dd</i>	5.12 <i>dd</i>	9'	2.45 <i>br q</i>	2.61 <i>br q</i>
7	2.79 <i>dddd</i>	2.66 <i>m</i>	10'	5.87 <i>m</i>	5.99 <i>br t</i>
8	6.68 <i>dd</i>	6.84 <i>dd</i>	12'	2.31 <i>m</i>	2.38 <i>m</i>
9	5.46 <i>d</i>	5.45 <i>d</i>	13' ₁	2.18 <i>m</i>	2.18 <i>m</i>
13 ₁	6.28 <i>d</i>	6.28 <i>d</i>	13' ₂	2.00 <i>m</i>	
13 ₂	5.81 <i>d</i>	5.88 <i>d</i>	15'	2.18 <i>m</i>	2.24 <i>qq</i>
14	—	9.48 <i>d</i>	16'	1.00 <i>d</i>	1.05 <i>d</i>
15	2.03 <i>d</i>	2.10 <i>br s</i>	17'		
18 ₁	6.18 <i>br s</i>	6.17 <i>br s</i>	19'	4.57 <i>br s</i>	4.58 <i>br s</i>
18 ₂	5.87 <i>br s</i>	5.87 <i>br s</i>	20'	1.79 <i>d</i>	1.77 <i>br s</i>
19	{ 4.32 <i>br d</i> 4.27 <i>br d</i>	4.28 <i>br s</i>	21' ₁	4.74 <i>br s</i>	4.76 <i>br s</i>
OAc	1.95 <i>s</i>	—	21' ₂	4.61 <i>br s</i>	4.68 <i>br s</i>

*OMe 3.81 *s*; †OAc 2.08, 2.06 *s*.

J[Hz]: Compounds **4** and **5**: 1, 2₁ = 5, 6 = 6, 7 = 10; 1, 2₂ = 7.5; 2₁, 2₂ = 2₁, 3₁ = 3₁, 3₂ ~ 13; 5, 15 = 1; 7, 8 ~ 1.5; 7, 13₁ = 3.3; 7, 13₂ = 3; 8, 9 = 8.5; 19, 19' = 15; compounds **5** and **6**: 1', 2' = 5', 6' = 8', 9' = 9', 10' = 7; 2', 20' = 1.5; 15', 16' = 15', 17' = 6.5.

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