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SESQUITERPENOID AND ACETYLENIC CONSTITUENTS OF SEVEN *CLIBADIUM* SPECIES

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Key Word Index—*Clibadium* cf. *asperum*; *C. erosum*; *C.* cf. *glomeratum*; *C. grandifolium*; *C. leiocarpum*; *C. pilonicum* and *C. surinamense*; Compositae; Heliantheae; new germacrolide type sesquiterpene lactone; new acetylenic alcohol; new sesquiterpene hydrocarbon.

Abstract—The investigation of seven *Clibadium* species resulted in the isolation of a new germacrolide from *C. surinamense*, *trans*-β-bergamotene, a new sesquiterpene from *C. asperum* and a new C-17 acetylenic alcohol from *C. glomeratum*. Five known acetylenic compounds were present in some members of the genus. In good agreement with morphological data, the presence of ichthyothereol and its acetate in several members of *Clibadium* suggest affiliation with the genus *Ichthyothere* within the subtribe Milleriinae (Heliantheae-Compositae).

INTRODUCTION

In a biochemical systematic study of the subtribe Melampodiinae [1, 2] we have analysed the herbaceous parts of several *Clibadium* L. species to learn about the type of chemical constituents within this genus and, from the chemical data, its position within the subtribe. Previously, the isolation of the acetylenic compounds ichthyothereol (**6**) and its acetate (**7**) from *Ichthyothere terminalis* (Spreng.) Blake [3] and *Clibadium sylvestre* (Aubl.) Baill. [4] has been reported. In this paper the results of a phytochemical screening of seven *Clibadium* species are described.

RESULTS AND DISCUSSION

Roots of *C. surinamense* from Ecuador contain the known acetylenic compounds **4**, **5** [11], ichthyothereol (**6**) and its acetate (**7**) [3, 4]. Aerial parts provided, besides **6**, **7** and **8**, germacrene D (**1**) and caryophyllene (**2**). Aerial parts of *C. surinamense* from Costa Rica produce in small amounts a new sesquiterpene lactone, $C_{15}H_{20}O_3$ (MS) mp 139° (polym.). A strong UV end absorption together with an IR signal at 1770 cm^{-1} and an NMR spectrum with sharp doublets at 5.54 and 6.28 ppm were indicative of an α,β-unsaturated γ-lactone. Further NMR signals exhibited absorptions typical for a germacrolide [5], represented by **3a**, the assignments of which are summarized in Table 1.

Table 1. ^{1}H NMR parameters* of compounds **3a** and **3b**

	3a	**3b**
H-1	5.01 *brdd* (11.0; 5.0)	6.26 *dd*(11.0; 5.0)†
H-5	4.81 *brd* (10.0)	4.99 *brd* (10.5)
H-6	4.55 *dd* (10; 9)	4.44 *dd*(10; 9)
H-7	2.60 *m*	3.06 *m*
H-13a	5.54 *d* (3.2)	5.54 *d* (3.0)
H-13b	6.28 *d* (3.8)	6.28 *d* (3.5)
C-4-Me	1.62 *d* (1.2)	1.56 *brs*
H-14a	3.80 *d* (12)	9.95 *s*
H-14b	4.23 *d* (12)	

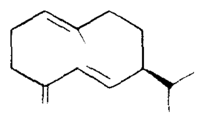
*Run at 270 MHz in $CDCl_3$ with TMS as internal standard. Chemical shifts are given in δ values in ppm. Multiplets are given by the usual symbols. Figures in parentheses are coupling constants or line separations in Hz.

†Partially obscured by H-13b.

The presence of only one vinyl methyl signal, a doublet at 1.62 ppm, together with the appearance of two geminally coupled proton signals at 3.80 and 4.23 ppm indicated an oxygen function at either C-14 or C-15. The presence of an OH group in **3a** was supported by an IR absorption at 3500 cm^{-1} and a M-18 peak at m/e 230 in the mass spectrum. Evidence for the position of the OH group at C-14 was provided by oxidation of **3a** with activated MnO_2, which led to the aldehyde **3b** with an H-1 absorption at 6.26 ppm compared to 5.01 ppm in **3a**. Furthermore, the position of H-1 and the H-14 aldehyde proton absorption at 9.95 ppm in **3b** provided evidence for the presence of a 1(10)-*trans* double bond [6]. We have previously isolated the 15-hydroxy isomer from *Platycarpha glomerata* [12] and other taxa of the Compositae. The NMR data of the two isomeric germacranolides and the corresponding aldehydes differ distinctly.

On biogenetic grounds, the configuration at C-7 in **3a** was assumed to contain an α-hydrogen. The large coupling constants ($J_{5,6} = 10$ Hz, $J_{6,7} = 9$ Hz) indicated antiperiplanar orientations of H-7, H-6 and H-5 suggesting a *trans*-lactone (H-6β) and a *trans*-4,5-double bond with H-5 being placed below the plane of the medium ring as previously shown for other germacrolides with a crown-conformation of the ten-membered ring [7].

Extracts of aerial parts of *C.* cf. *asperum* provided squalene, caryophyllene (**2**), β-sesquiphellandrene (**10**) [8] and two known acetylenic compounds (**8** and **9**) [11]. Nonpolar fractions, after repeated PLC on $AgNO_3$-



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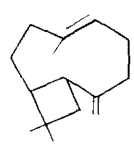

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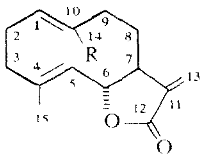


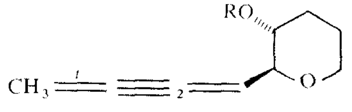
3a R = CH_2OH
3b R = CHO

$CH_3{=}{\equiv}_4{=}$

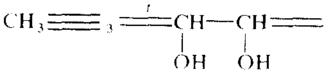
4

$CH_3\overset{t}{=}{\equiv}_2\overset{tt}{=}_2(CH_2)_5OAc$

5

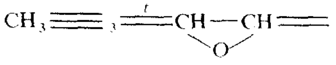


6 R = H
7 R = Ac



$CH_3{\equiv}_3\overset{t}{=}\underset{OH}{CH}{-}\underset{OH}{CH}{=}$

8



$CH_3{\equiv}_3\overset{t}{=}CH{-}CH{=}$ (with O bridging the two CH)

9

impregnated Si gel, gave a new sesquiterpene hydrocarbon, $C_{15}H_{24}$ (MS) the NMR and mass spectral data of which were in good agreement with a carbon framework represented by β-bergamotene [9]. In accordance with the presence of a dimethylallyl moiety as in **13**, a base peak at *m/e* 69 was observed. Vinyl methyl absorptions at 1.61 and 1.71 ppm, an olefinic proton at 5.16 ppm (H-10) and a broadened quartet at 1.95 ppm (2H-9) verified the mass spectral assignment. However, reported NMR data [9] for *cis*-β-bergamotene (**13**) differed distinctly from those of the newly isolated sesquiterpene. In addition, an $[\alpha]_D$ +35.8° had been reported for **13** whereas the new compound showed a negative value ($[\alpha]_D$ −3.1°) as previously observed for (−)-*trans*-α-bergamotene (**14**) [10]. The NMR signals assigned to the bicyclic portion of the new compound closely resembled the parameters of d-β-pinene (**11**), suggesting a skeletal arrangement in which the side chain is oriented away from the bicyclic ring as in **14** (see Table 2). In analogy to previous correlations between (−)-α-pinene and **14** [10] the new compound was assigned structure **12** which we named (−)-*trans*-β-bergamotene.

Aerial parts of *C.* cf. *glomeratum* contained squalene and mixtures of fatty acids and esters. In addition a new acetylenic alcohol, $C_{17}H_{22}O$ (MS) was isolated. The UV spectrum suggested the presence of a diene–diine chromophore. Mass spectral peaks at *m/e* 213 and 41 indicated the loss of C_2H_5 (29 mu) and $CH_2{=}CH{-}CH_2$ (41 mu), respectively, most likely due to scissions near the terminal positions. The base peak at *m/e* 145 was assigned the ion generated by the loss of $C_6H_{11}O$ (99 mu) resulting from a cleavage between C-6,7 in **15**. Accommodation of the above data combined with the 270 MHz NMR parameters resulted in structure **15** for the new acetylenic alcohol.

Extracts of aerial parts of *C. grandifolium*, *C. leiocarpum* and *C. pilonicum* contained squalene, fatty acids and derived esters. *Clibadium erosum* produced, besides major amounts of oleic and linoleic acid, ichthyothereol (**6**) and the acetate (**7**). A crystalline mixture of two sterols ($C_{29}H_{48}O$ and $C_{29}H_{50}O$ by MS) was obtained which could not be separated; therefore, no further investigations were carried out.

Although detailed morphological and cytological investigations on *Clibadium* are still in progress [13], the chemical data presented here are sufficiently interesting that several comments on their systematic import are warranted. The chemistry bears upon: (1) generic and subtribal relationships, and (2) specific and infraspecific affinities.

As for generic affinities, the isolation of ichthyothereol and its acetate from *C. erosum* and *C. surinamense*, in relation to these same compounds from *Ichthyothere terminalis* [3], suggests close ties between the two genera. At one time they had been treated as belonging to separate subtribes of the Heliantheae [14, 15], but more recently the two taxa have been recognized as being closely related and belonging to the same subtribe [1,2,15]. Previous chemical studies had shown only that *C. sylvestre* [4] and *I. terminalis* [3] shared ichthyothereol and its acetate. The present studies strengthen the taxonomic relationship between the two genera by showing the presence of these compounds in two additional species of *Clibadium*.

With regard to subtribal relationships, both *Clibadium* and *Ichthyothere* lack melampolides, a type of sesquiterpene lactone characteristic of *Melampodium* L. [17] and *Polymnia* L. [18] of the subtribe Melampodiinae [2]. This suggests that the most recent placement of the former two genera in a different subtribe, the Milleriinae [2], may be justified. Previous interpretations placed the two genera together in the Melampodiinae [16].

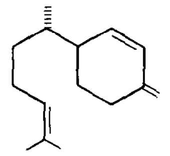
Several interesting specific and infraspecific relationships can be noted. Ichthyothereol and its acetate are now known in *Clibadium sylvestre*, *C. surinamense*, and *C. erosum*. This suggests a close evolutionary tie among the three species, and this is borne out by the

Table 2. 270 MHz NMR parameters of compounds **11*** and **12** in $CDCl_3$

	11	**12**	(Hz)
H-1	2.46 *t*	2.53 (2.65)† *t*	1,6β = 5.5
H-3α	2.55 *ddd* (*br*)	2.56 (2.54) *ddd* (*br*)	1,5 = 5.5
H-3β	2.25 *ddd*	2.25 *ddd* (*br*)	3α,3β = 17
H-4	1.84 *m*	1.84 *m*	5,6β = 5.5
H-5	1.97 *ddt*	2.07 *ddt*	6α,6β = 10
H-6α	1.42 *d*	1.43 (1.43) *d*	9,10 = 7
H-6β	2.31 *dt*	2.30 (2.29) *dt*	10,12 = 1
H-8	1.24 *s*	1.61 *m*	10,13 = 1
H-9	—	1.95 (2.07 *q* (*br*)	
H-10	—	5.16 (5.30) *t* (*br*)	
H-12	—	1.71 (1.75) *s* (*br*)	
H-13	—	1.61 (1.63 *s* (*br*)	
H-14	0.72 *s*	0.72 (0.82) *s*	
H-15a	4.63 *m*	4.63 (4.81) *m*	
H-15*b*	4.56 *m*	4.56 (4.77) *m*	

*Numbering analogue to **12**.

†Numbers in parentheses represent chemical shifts in C_6D_6.



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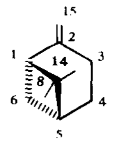
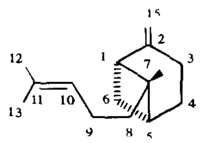


11*



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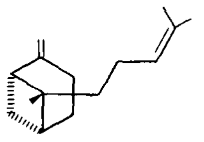

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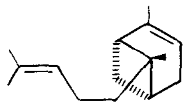

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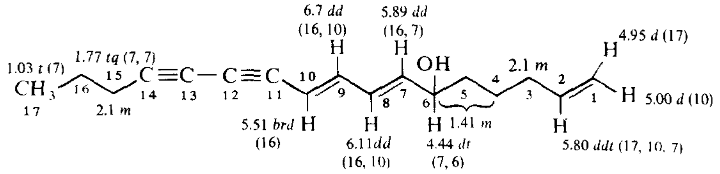

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* Numbering analogue to **12** for NMR comparison.

morphological studies. *Clibadium sylvestre* (= *C. asperum*) and *C. surinamense* are sufficiently close that an analysis of herbarium material indicates that hybridization apparently is occurring in Panama where the two species grow together [19]. *Clibadium erosum* is isolated geographically from the other two species in Mexico and the Caribbean, but morphologically it is similar to the other two taxa.

Because *Clibadium asperum* (= *C. sylvestre*) has ichthyothereol and its acetate [4], it would be suspected that the newly analysed collection of *C.* cf. *asperum* (SG 4508) would also have these compounds. This is not the case. Two factors need to be considered, however, in attempting to explain this difference. First, the voucher for the recent collection differs from typical *C. asperum* in having smaller and less congested flowering heads and narrower leaves, which suggests that it could be varietally or even specifically distinct. Second, infraspecific variation of the presence of ichthyothereol and its acetate is now known to occur in *C. surinamense* (i.e. these compounds were found in only one, King 6871, of the two collections analysed), and it is possible that such infraspecific chemical variation may occur also in *C. asperum*.

The preliminary chemical data, especially the polyacetylenes, indicate good potential for helping understand systematic relationships within *Clibadium* as well as with *Ichthyothere*. They also suggest that it would be worthwhile to examine species of *Desmanthodium* Benth. of Mexico and Central America, which is believed on morphological grounds also to be related to *Clibadium* [2]. Furthermore, the non-uniform occurrence of sesquiterpenoid compounds and acetylenes within at least some species of *Clibadium* indicates that populational surveys should be carried out on all species, so that infraspecific chemical variation can be effectively understood and dealt with taxonomically.

EXPERIMENTAL

IR: Beckman IR9, CCl_4; ^1H-NMR: Bruker WH270; MS: Varian MAT 711, 70 eV, direct probe; optical rotation: Perkin Elmer polarimeter, $CHCl_3$. Extractions of the air-dried, ground plant material of the *Clibadium* collections (vouchers at Ohio State University Herbarium [OS], Columbus, Ohio, except King and Garvey 6871 at the U.S. National Herbarium [US], were carried out with a 2:1 mixture of Et_2O–petrol ether (PE). The crude extracts were taken up in *ca* 20 ml MeOH and stored for 1 hr at $-20°$. Filtration of the fatty precipitates and evaporation of the filtrate gave a mixture of the crude oils. Crude fractionations were performed by CC (Si gel, activity II), beginning with PE followed by PE–Et_2O mixtures with increasing amounts of Et_2O (5, 10, 15, 25, 50, 100% Et_2O). Subsequent preparative layer chromatography (PLC, Si gel GF 254) of the crude fractions, which were monitored by TLC and ^{1}H NMR, were carried out with Et_2O–PE mixtures as eluants.

Clibadium surinamense L. (collected in Ecuador, Canar, *ca* 77 km ESE of Guajaquil, 22 Jan. 1976, King and Garvey 6871): 96 g of root material provided 1 mg **4**, 1 mg **5**, 1 mg **6** and 2 mg **7**. Extraction of 160 g of aerial parts gave 20 mg **1**, 20 mg **2**, 25 mg **5**, 5 mg **6** and 2 mg **7**. *Clibadium surinamense* (collected in Costa Rica, Alajuela, *ca* 9 miles N of Zarcero, 1 Nov. 1976, Stuessy and Gardner 4461): 300 g of aerial parts gave 0.8 g of crude oil which consisted mainly of oleic and linoleic acid (NMR). Repeated PLC (Et_2O) gave 5 mg **3a**, mp 139° (polymerization); UV, strong end absorption; IR bands at 3610 (sharp), 3500 (OH), 1770 (Me_2CO), 1665 (double bonds). The low resolution MS exhibited significant peaks at *m/e* (rel. int., assignment): 248 (6.0, M^+), 233 (8.0, $M-CH_3$), 230 (30, $M-H_2O$), 215 (24, $M-CH_3-H_2O$), 117 (45, C_9H_9), 105 (85, C_8H_8), 91 (98, C_7H_7), 81 (100). (Calc. for $C_{25}H_{20}O_3$: MW, 248.1412. Found: MW (MS), 248.1413).

Aldehyde **3b**. In 3 ml Et_2O, 3 mg **3a** were shaken with activated MnO_2 for 15 min, at room temp. Filtration and evapn gave **3b**, IR bands at 1760 (lactone), 1725 (C=O), and 1670 (double bonds). The low resolution MS showed significant peaks at *m/e* (rel. int., assignment): 246 (34, M^+), 228 (20, $M-H_2O$), 217 (20, $M-CHO$), 215 (26), 119 (34, C_9H_{11}), 117 (20, C_9H_9), 105 (50, C_8H_8), 91 (67, C_7H_7), 81 (100), 71 (50), 69 (74), 57 (78), 55 (84), 53 (67), 43 (61), and 41 (82). (Calc. for $C_{15}H_{18}O_3$: MW, 246.1256. Found: MW (MS), 246.1253).

Clibadium cf. *asperum* (Aubl.) DC. (collected in Costa Rica, Puntarenas, 14 miles SE of Piedras Blancas, 5 Nov., 1976, Stuessy and Gardner 4508): 300 g of aerial parts gave, besides 85 mg squalene, 5 mg **2**, 13 mg **12**, 15 mg **8**, 26 mg **9**, 25 mg **10**.

trans-β-Bergamotene (**12**): Repeated PLC on $AgNO_3$-impregnated Si gel (E/PE 1:10) of nonpolar CC fractions provided 13 mg **12**; colorless oil; $[\alpha]_D^{20}$ $-3.1°$ (*c* 0.39, $CHCl_3$). IR: 3080, 1640 and 875 cm^{-1} ($=CH_2$). MS: M^+, *m/e* (rel. intensity) 204 (23); $M-CH_3$, 189 (20); $M-C_3H_7$, 161 (44); $C_5H_9^+$, 69 (100). (Calc. for $C_{15}H_{24}$: MW, 204.1878. Found: MW (MS), 204.1878.)

Clibadium cf. *glomeratum* Greenm. (collected in Costa Rica, Puntarenas, 18.5 miles N of Villa Neily, 6 Nov., 1976, Stuessy and Gardner 4519): 300 g of dried aerial parts provided 90 mg squalene and 4 mg **13** (DC, E/PE 1:3) besides mixtures of fatty acids and their esters.

Heptadeca-1,7*t*,9*t*-trien-11,13-diin-6-ol (**15**): colorless oil, UV $\lambda_{max}^{Et_2O}$ 306, 292, 237, 226 nm; IR (CCl_4): 3620 (OH), 2225 ($C\equiv C$), 1640, 980 ($-CH{=}CH-$, *t*,*t*), 3080, 915 cm^{-1} ($-CH{=}CH_2$); MS: M^+ *m/e* (rel. int.) 242.165 ($C_{17}H_{22}O$, 22%); $M-C_2H_5$, 213 (24); $M-C_6H_{11}O$, 145 (100); $CH_2{=}CH-CH_2^+$, 41 (51). NMR spectral assignments are given in formula **15**.

Clibadium grandifolium S. F. Blake (collected in Panama, Coclé, 3 km N of El Valle de Antón, 4 Jul., 1976, Hartman 3936); *Clibadium leiocarpum* Steetz in Seemn. (collected in Costa Rica, Alajuela, *ca* 9 miles N of Zarcero, 1 Nov., 1976, Stuessy and Gardner 4462) and *Clibadium pilonicum* Stuessy (collected in Panama, Coclé, W ridge near summit of Cerro Gaital, 10 Jul., 1976, Hartman 3963): 300 g of the dried aerial parts of the above 3 collections contained 20 mg, 10 mg and 15 mg of squalene, respectively, besides various amounts of fatty acids and their esters.

Clibadium erosum DC. (Collected in Mexico, Oaxaca, 11.8 miles W of Tuxtepec, 16 Nov., 1976, Stuessy and Gardner 4574): 280 g of herbaceous parts provided, besides major amounts of oleic and linoleic acid (identified by 270 MHz ^{1}H(NMR), 8 mg of **6** (PLC, Et_2O–PE, 1:1) which was identified by UV, NMR and MS. Less polar fractions contained traces of the acetate **7**. After treatment with CH_2N_2 (esterification by fatty acids) PLC (Et_2O–PE, 4:1, 2 runs) gave 15 mg of a mixture of sterols [MS, *m/e* 412.3701 ($C_{29}H_{48}O$) and *m/e* 414] which could not be separated and were therefore not further investigated.

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