The Pseudocalanolides: Structure Revision of Calanolides C and D

ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · JULY 1995

Impact Factor: 3.8 · DOI: 10.1021/np50120a015 · Source: PubMed

4 AUTHORS, INCLUDING:

Tawnya C Mckee

National Institutes of Health

92 PUBLICATIONS **2,477** CITATIONS

SEE PROFILE

Journal of Natural Products
Vol. 58, No. 6, pp. 916-920, June 1995

THE PSEUDOCALANOLIDES: STRUCTURE REVISION OF CALANOLIDES C AND D

TAWNYA C. MCKEE, JOHN H. CARDELLINA II, GEOFFREY B. DREYER,[1] and MICHAEL R. BOYD*

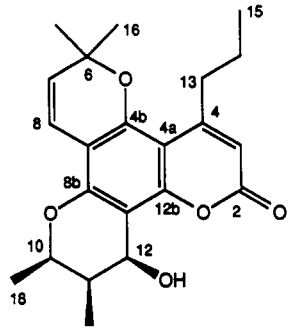
Laboratory of Drug Discovery Research and Development, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Building 1052, Room 121, Frederick, Maryland 21702-1201

ABSTRACT.—Nmr spectra of synthetic structures corresponding to those initially reported for natural compounds calanolide C [**1**] and calanolide D [**2**] showed some subtle differences from those of the natural products. Further analysis has resulted in revision of the structures of the natural compounds, now renamed pseudocalanolides C [**3**] and D [**4**]. The absolute stereochemistry of pseudocalanolide C was established as [6*S*,7*S*,8*R*] using the modified Mosher's method.

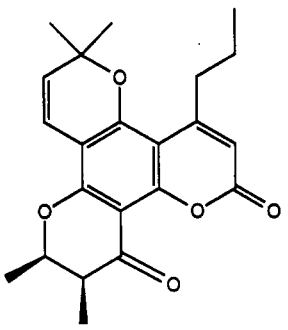
The isolation of the calanolides, a novel class of HIV-1 reverse transcriptase inhibitors, from a tropical rainforest tree, *Calophyllum lanigerum* Miq. var. *austrocoriaceum* (T.C. Whitmore) P.F. Stevens (Guttiferae), was recently described (1). The isolation of some related anti-HIV-active dipyranocoumarins, the inophyllums, from *Calophyllum inophyllum* L. was the subject of another report (2). Subsequently, the total syntheses of racemic calanolides A [**5**], B, C [**1**], and D [**2**] were published (3). However, in the latter paper there was an apparent discrepancy in the ^{1}H-nmr spectrum of synthetic calanolide C compared to that of the natural product. The natural compound had an observed chemical shift of δ 6.83 for H-8 (1), while the corresponding proton resonance of the synthetic material reportedly was observed at δ 6.63 (3). Our direct comparison of recent ^{1}H-nmr spectra of natural compounds originally identified as calanolides C and D and of the synthetic, racemic calanolides C and D has confirmed this discrepancy. Accordingly, we communicate here the structure revision of the two natural compounds to **3** and **4**, respectively, where the orientation of the two pyran rings is opposite that initially reported.

The primary structural challenge of the dipyranocoumarin class lies in the fully substituted central aromatic ring. Three of the positions on the ring are substituted with oxygen and have similar carbon chemical shifts (e.g., δ 151.1, 153.1, 154.4 in calanolide A). The chemical shifts of the remaining three carbons are also very similar (δ 104.0, 106.3, 106.3 for calanolide A). The assignment of these chemical shifts to individual carbons can only be accomplished via long-range heteronuclear correlation experiments such as HMBC. However, multiple correlations to three of these carbons are necessary to distinguish between the two possible ring configurations exemplified by the calanolide C [**1**] and pseudocalanolide C [**3**] structures. The assignment of the central ring carbons is further complicated in calanolide D [**2**] and pseudocalanolide D [**4**] by the ketone functionality, which decreases the number of observable correlations to the central ring. In the original report of the natural compounds (1), the carbon signals for "calanolide C" (now pseudocalanolide C) were assigned by analogy to calanolide A [**5**] and for "calanolide D" (now pseudocalanolide D) using limited data from a single HMBC experiment ($^nJ_{ch}$=8.3 Hz), plus analogy to **5** and chemical correlation to "calanolide C." A reanalysis of the original HMBC data indicated that the observed correlations did not distinguish between the two pos-

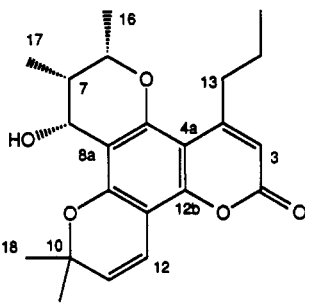
[1]SmithKline Beecham Pharmaceuticals R & D, King of Prussia, PA 19406; current address: ONYX Pharmaceuticals, 3031 Research Drive, Richmond, CA 94806.



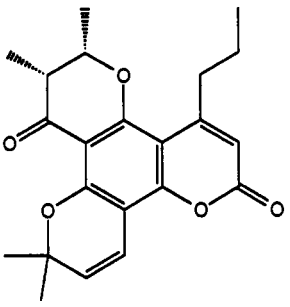
1



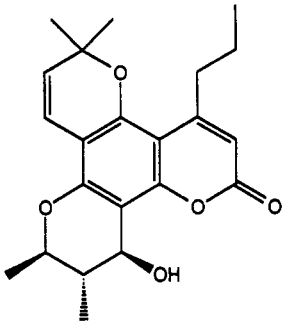
2



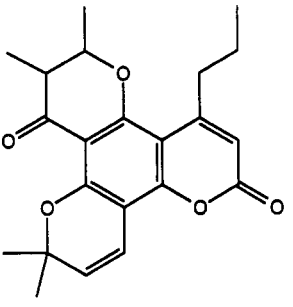
3



4



5



6

sible ring systems. Further HMBC experiments optimized for 8.3 and 5.5 Hz long-range coupling constants have been conducted for pseudocalanolide C. Observed correlations to δ 2.79 and 2.88 (H-13, -13′) from C-3 (δ 111.1) and C-4 (δ 158.6) were consistent with those observed for calanolides A and B. An additional correlation between δ 103.5 and these same protons led to its assignment as C-4a. Observed correlations to H-7 (δ 2.22) and H-8 (δ 5.06) from a carbon at δ 109.2 suggested its placement at C-8a. In the same manner, the carbon at δ 154.6 was assigned to C-8b based on its correlations to H-8 (δ 5.06) and H-12 (δ 6.83); δ 152.6, which correlated to H-8 and H-6 (δ 4.32), was assigned to C-4b; and δ 150.6 was assigned to C-12b, because it correlated to H-12. This left the carbon signal at δ 102.9 for C-12a. Thus, structure **3** was established for pseudocalanolide C.

The relative stereochemistry of pseudocalanolide C is the same as that originally reported for "calanolide C" (1). The absolute stereochemistry of pseudocalanolide C was determined using the modified Mosher's method (4,5). By this method (see Figure 1), the absolute configuration of C-8 was determined to be *R*, thus establishing the absolute configuration of **3** to be [6*S*,7*S*,8*R*]. There

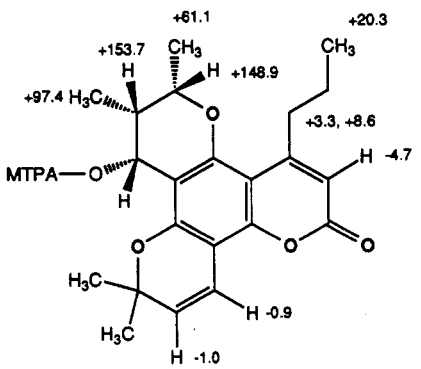


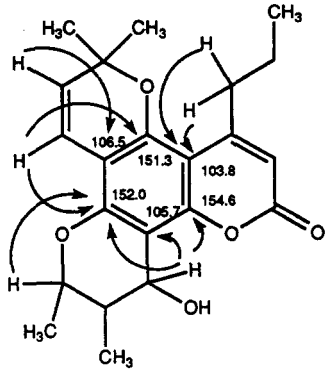
FIGURE 1. ^{1}H-nmr Δδ values (Δδ=δ_S-δ_R in Hz at 500 MHz) for (*R*)- and (*S*)-MTPA esters of pseudocalanolide C [**3**].

was essentially no difference in the coupling constants of H-6, H-7, or H-8, suggesting that the ring conformation was unchanged by esterification.

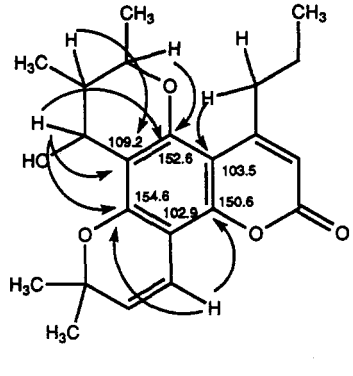
An HMBC experiment ($^{n}J_{ch}$=5.5 Hz) was also performed for synthetic calanolide C. Observed correlations to δ 2.92 and 2.86 (H-13,13′) from C-3 (δ 110.1) and C-4 (δ 158.9) were consistent with assignments for the other calanolides. Additional correlations between δ 103.8 and H-13 and H-3 (δ 5.94) placed this carbon at C-4a. The carbon signal at δ 152.0 was correlated to three protons [δ 6.63 (H-8), 4.39 (H-10), and 5.09 (H-12)] and, therefore, had to be placed at C-8b. H-12 (δ 5.09) was also correlated to carbons at δ 105.7 (C-12a) and δ 154.6 (C-12b), while H-8 (δ 6.63) showed additional correlations to a carbon at δ 151.3 (C-4b) and H-7 (δ 5.54) was correlated to C-8a (δ 106.5). These observed correlations are analogous to those observed for calanolides A and B and are best accounted for by structure **1**. This structure is identical to that originally proposed for calanolide C. Figure 2 illustrates the critical correlations in both **1** and **3**.

As with pseudocalanolide C, HMBC experiments optimized for $^{n}J_{CH}$=8.3 and 5.5 Hz were obtained for pseudocalanolide D. These experiments allowed the unequivocal assignment of all but two carbons. The assignments for those carbons could not be firmly established because of a paucity of the compound and its instability in $CDCl_3$. The uncertainty in the assignment of C-8b and C-12b is reflected in Table 1. Pseudocalanolide D has the same gross structure as tomentolide B [**6**], reported as a racemate from the seeds of *Calophyllum tomentosum* (6). However, based on differences between the reported ^{1}H-nmr data of tomentolide B and pseudocalanolide D, we believe that they are diastereomers. As noted in the original report (1), pseudocalanolide D can be cleanly converted to pseudocalanolide C by reduction with $NaBH_4$, and, therefore, must have structure **4** and the 6*S*,7*R* stereochemistry,[2,3]

The nmr keys to correct placement of the pyran rings in this class of compounds are the ^{1}H-nmr shifts for the olefinic proton at H-8/H-12, where a distinctive 0.2 ppm shift difference is typically observed (see Table 2), and the ^{13}C-nmr



1



3

FIGURE 2. Key HMBC correlations for the assignments of the benzene ring carbons of synthetic calanolide C [**1**] and natural pseudocalanolide C [**3**].

[2]After completion of this work, Palmer and Josephs reported an independent racemic synthesis of structures **1** and **2** and proposed the same structure revision for the natural compounds (8).

[3]It should be noted that the structures originally assigned (1) to calanolides A and B are correct, as demonstrated by rigorous spectral analyses (1) and synthesis (3).

TABLE 1. ^{13}C-Nmr Data ($CDCl_3$) for Compounds **1–4**.

Carbon	Compounds			
	1[a]	**2**[a]	**3**	**4**
2	160.2	159.7	160.8	160.0
3	110.1	112.1	111.1	111.4
4	158.9	157.0	158.6	158.1
4a	103.8	104.0	103.5	102.7
4b	151.3	155.8[b]	152.6	160.0
6	77.6	79.2	75.6	77.4
7	126.9	126.9	35.1	45.7
8	116.6	115.9	65.9	192.9
8a	106.5	105.5	109.2	106.8
8b	152.0	158.8[b]	154.6	157.6[c]
10	75.7	77.3	78.8	78.9
11	35.2	45.9	126.9	128.2
12	63.0	191.5	115.7	115.0
12a	105.7	102.9	102.9	104.1
12b	154.6	156.0	150.6	154.3[c]
13	38.7	38.8	38.9	38.9
14	23.3	23.2	23.2	23.0
15	14.0	13.9	14.0	13.9
16	27.8	38.1	16.8	15.9
17	27.8	28.2	7.2	9.0
18	16.2	16.1	28.2	28.0
19	9.6	9.2	28.4	28.1

[a]Synthetic **1** and **2** (3).

[b]Correlations were observed to both carbons from H-8, assignments were based on an additional very weak correlation from H-10 to C-8a.

[c]Assignments may be interchangeable.

shifts for C-8/C-12 and C-8a/C-12a, where complementary 3 ppm shifts are observed (see Table 1).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—As previously reported (7).

PLANT MATERIAL.—As previously reported (1).

EXTRACTION AND ISOLATION.—As previously reported (1).

SYNTHESIS OF **1** AND **2**.—As previously reported (3).

Pseudocalanolide C [**3**].—$[\alpha]_D$ +68° (c=0.7, $CHCl_3$); ir (film) ν max 2960, 1729, 1620, 1582, 1120 cm^{-1}; hreims observed m/z 370.1695 (calcd for $C_{22}H_{26}O_5$, 370.1780); lreims m/z 370 (M^+, 25), 355 (100), 337 (25), 299 (35).

Pseudocalanolide D [**4**].—$[\alpha]_D$ +60° (c=0.5, $CHCl_3$); ir (film) ν max 2960, 1734, 1697, 1684, 1575, 1558 cm^{-1}; hreims observed m/z 368.1213 (calcd for $C_{22}H_{24}O_5$, 368.1624); lreims m/z 368 (M^+, 25), 353 (100), 297 (68).

Preparation of the (R)- and (S)-MTPA esters of pseudocalanolide C.—A solution of (*R*)-α-methoxy-α-(trifluoromethyl)-phenylacetic acid chloride (2.7 mg in 50 μl of C_6H_6) was added to 3.1 mg pseudocalanolide C [**3**] in 4.5 ml C_6H_6. A small amount of (dimethylamino)pyridine (DMAP) and 10 μl of triethylamine were added and the reaction was refluxed at 90°. After 6 h, an additional 3 mg of (*R*)-MTPA chloride was added and the reaction was refluxed overnight. The mixture was cooled and then chromatographed on a short silica plug (1×1 cm) eluting stepwise with a gradient of hexane/EtOAc. The desired (*S*)-MTPA ester eluted with 20% EtOAc. The same procedure was used to generate the (*R*)-MTPA ester from the (*S*)-MTPA chloride.

ACKNOWLEDGMENTS

We thank Ms. Heidi Bokesch for technical support on this project.

LITERATURE CITED

1. Y. Kashman, K.R. Gustafson, R.W. Fuller, J.H. Cardellina II, J.B. McMahon, M.J. Currens, R.W. Buckheit, Jr., S.H. Hughes, G.M. Cragg, and M.R. Boyd, *J. Med. Chem.*, **35**, 2736 (1992).
2. A.D. Patil, A.J. Freyer, D.S. Eggleston, R.C.

TABLE 2. Comparison of ^{1}H-Nmr Data ($CDCl_3$) for Compounds **1–4**, and **6**.

Proton	Compounds				
	1[a]	**3**	**2**[a]	**4**[b]	**6**[c]
3	5.94, s	5.94, t, J=1.0 Hz	6.02, s	5.98, t, J=1.0 Hz	5.79, s
6	—	4.32, dq, J=2.5, 7.0 Hz	—	4.69, dq, J=3.0, 6.5 Hz	4.22, m
7	5.54, d, J=10 Hz	2.22, ddq, J= 2.5, 6.0, 7.0 Hz	5.57, d, J=10.0 Hz	2.61, dq, J=3.0, 7.0 Hz	2.42, m
8	6.63, d, J=10 Hz	5.06, dd, J=6.0, 1.5 Hz	6.63, d, J=10.0 Hz	—	—
10	4.39, dq, J=6.7, 3.3 Hz	—	4.67, dq, J=7.0, 3.5 Hz	—	—
11	2.28, m	5.56, d, J=10.5 Hz	2.66, dq, J=7.0, 3.0 Hz	5.61, d, J=11.0 Hz	5.58, d, J=10 Hz
12	5.09, dd, J=6.0, 1.0 Hz	6.83, d, J=10.5 Hz	—	6.78, d, J=11.0 Hz	6.85, d, J=10 Hz
13,13′	2.92, m 2.86, m	2.88, ddd 2.77, ddd	2.85, dt, J=7.0, 2.5 Hz	2.85, m	2.81, t
14,14′	1.66, m	1.60, sext, J=7.0 Hz	1.61, sext, J=7.5 Hz	1.63, sext, J=7.0 Hz	nd[d]
15	1.04, t, J=7.0 Hz	0.98, t, J=7.5 Hz	1.00, t, J=7.5 Hz	1.01, t, J=7.5 Hz	1.00, t
16	1.49, s	1.41, d, J=7.0 Hz	1.52, s	1.42, d, J=7.0 Hz	1.53, d, J=7 Hz
17	1.49, s	1.06, d, J=7.5 Hz	1.51, s	1.14, d, J=7.5 Hz	1.15, d, J=7 Hz
18	1.42, d, J=7.0 Hz	1.46, s	1.39, d, J=7.0 Hz	1.50, s	1.51, s
19	1.14, d, J=7.0 Hz	1.46, s	1.13, d, J=7.5 Hz	1.50, s	1.51, s
OH	3.29, d, J=1.0 Hz	3.64, d, J=1.5 Hz	—	—	—

[a]Synthetic **1** and **2** (3).
[b]Kashman *et al.* (1).
[c]Nigami and Mitra (6).
[d]Not reported.

Haltiwanger, M.F. Bean, P.B. Taylor, M.J. Caranfa, A.L. Breen, H.R. Bartus, R.K. Johnson, R.P. Hertzberg, and J.W. Westley, *J. Med. Chem.*, **36**, 4131 (1993).
3. B. Chenera, M.L. West, J.A. Finkelstein, and G.B. Dreyer, *J. Org. Chem.*, **58**, 5605 (1993).
4. I. Ohtani, T. Kusumi, M.O. Ishitsuka, and H. Kakisawa, *Tetrahedron Lett.*, **30**, 3147 (1989).
5. I. Ohtani, T. Kusumi, Y. Kashman, and H. Kakisawa, *J. Org. Chem.*, **56**, 1296 (1991).
6. S.K. Nigam and C.R. Mitra, *Tetrahedron Lett.*, 2633 (1967).
7. K.R. Gustafson, J.H. Cardellina II, J.B. McMahon, L.K. Pannell, G.M. Cragg, and M.R. Boyd, *J. Org. Chem.*, **57**, 2809 (1992).
8. C.J. Palmer and J.L. Josephs, *Tetrahedron Lett.*, **35**, 5363 (1994).

Received 4 October 1993