Phytochemistry, Vol. 25, No. 11, pp. 2661–2663, 1986.
Printed in Great Britain.

0031–9422/86 $3.00 + 0.00
Pergamon Journals Ltd.

DEOXYCOLLYBOLIDOL, A SESQUITERPENE FROM *COLLYBIA PERONATA*

MATS FOGEDAL and THOMAS NORBERG

University of Stockholm, Department of Organic Chemistry, Arrhenius Laboratory, S-106 91 Stockholm, Sweden.

(*Revised received* 10 *March* 1986)

Key Word Index—*Collybia peronata*; Basidiomycetes; sesquiterpene; 3β-(3-furyl)-3,4,4aα,8β,9,9aα-hexahydro-5-β-methyl-5,8-methano-1*H*-pyrano[3,4-d]oxepin-1,6(5*H*)-dione; deoxycollybolidol.

Abstract—A crystalline compound, deoxycollybolidol, was isolated from the chloroform–methanol extracts of *Collybia peronata*. On the basis of elemental analysis and spectral data, the structure 3β-(3-furyl)-3,4,4aα,8β,9,9aα-hexahydro-5-β-methyl-5,8-methano-1*H*-pyrano[3,4-d]oxepin-1,6(5*H*)-dione is proposed.

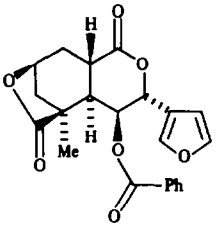
INTRODUCTION

In 1974, Bui *et al.* reported [1] the isolation of two sesquiterpenes, collybolide (**1**) and isocollybolide (**2**), of a new type from *Collybia maculata* (Basidiomycetes). We now report the isolation and structural elucidation of deoxycollybolidol, a related sesquiterpene from *Collybia peronata*.

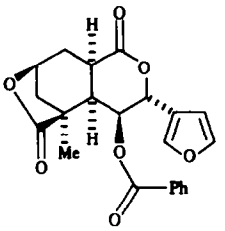
RESULTS AND DISCUSSION

Ground and lyophilized fruiting bodies of *C. peronata* were extracted with chloroform–methanol (2:1) and the extract was fractionated by silica gel column chromatography. A pure, crystalline material was isolated (49 mg from 35.5 g lyophilized material). The EI and FAB mass spectra both showed a molecular ion at *m/z* 276. ^{13}C NMR and ^{1}H NMR spectroscopy showed the presence of 15 carbons and 16 protons, respectively. These data, together with elemental analysis data, gave the molecular formula $C_{15}H_{16}O_5$. After assignment of the NMR spectra (Tables 1–3) the structural elements shown in Fig. 1 were evident.

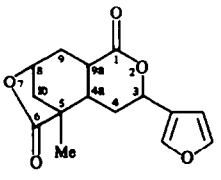
The IR spectrum showed strong carbonyl absorptions at 1745 and 1790 cm^{-1}, but there were no hydroxyl or carboxylic acid absorptions. The evidence presented above, together with consideration of biosynthetic principles, suggested a sesquiterpene dilactone structure such as **3** or **4**. Examination of dihedral angles of molecular models, which is appropriate for the conformationally rigid, bicyclic cyclohexane ring present in **1**–**4**, revealed that $\theta_{H\text{-}8,H\text{-}10(ax)}$ should be ~ 80° in **1**, **2** and **3**. Thus, the value of less than 1 Hz for $J_{H\text{-}8,H\text{-}10(ax)}$ is compatible with the structure **3**. Indeed, in collybolide and isocollybolide, $J_{H\text{-}8,H\text{-}10(ax)}$ is 0 and 1 Hz, respectively. Further comparison of NMR chemical shifts and ^{1}H coupling constants with those [1] of collybolide and isocollybolide shows strong similarities. The value of *J*4a, 9a, (8.6 Hz) agrees best with the value of isocollybolide (9.0–9.1), therefore H-4a and H-9a are *cis*. The values of $J_{H\text{-}4(ax),H\text{-}3}$ and $J_{H\text{-}4(eq),H\text{-}3}$ do not give definite proof of the configuration at C-3, since the conformation of this lactone ring is less predictable and the dihedral angles are therefore not evident for any of the C-3 epimers. It is assumed, however, that the conformation of this ring in solution resembles that found [2] for crystalline isocollybolide by X-ray crystallography. Then $\theta_{H\text{-}4(ax),H\text{-}3}$ and $\theta_{H\text{-}4(eq),H\text{-}3}$ for the two C-3 epimers are either 177° and 59° or 48° and 70°. The values of $J_{H\text{-}4(ax),H\text{-}3}$ (6.4 Hz) and $J_{H\text{-}4(eq),H\text{-}3}$ (4.4 Hz) are most compatible with



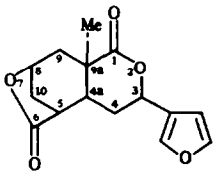
1



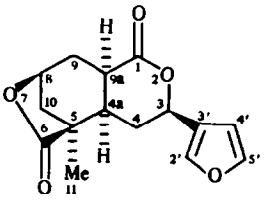
2



3



4



5

Table 1. ^{13}C NMR chemical shifts of deoxycollybolidol

Carbon	Chemical shift
11	18.9
9	28.7
4	29.1
9a	35.6
4a	39.3
5	43.3
10	44.1
3	71.4
8	74.5
4′	108.6
3′	124.7
5′	139.3
2′	144.2
1	171.2
6	178.5

Table 2. ^{1}H NMR chemical shifts of deoxycollybolidol

H	Chemical shift
11	1.29
9 (ax)	1.78
10 (ax)	1.83
4 (ax)	2.22
10 (eq)	2.32
4 (eq)	2.42*
4a	2.47*
9a	2.90
9 (eq)	3.05
8	4.78
3	5.63
4′	6.39
5′	7.42
2′	7.42

*Determined by spin simulation.

Table 3. Proton–proton coupling constants for deoxycollybolidol

Protons	Coupling constant (Hz)
3, 4 (ax)	6.4
3, 4 (eq)	4.4*
3, 2′	0.1–0.5
4 (ax), 4 (eq)	14.2
4 (ax), 4a	6.4
4 (eq), 4a	7.2*
4a, 9a	8.6
8, 9 (ax)	1.4
8, 9 (eq)	4.2
8, 10 (ax)	< 1
8, 10 (eq)	6.4
9 (ax), 9a	8.6
9 (eq), 9a	1.8
9 (ax), 9 (eq)	14.8
9 (eq), 10 (eq)	1.8
10 (ax), 10 (eq)	11.8
2′, 4′	1.4
4′, 5′	1.4

*Determined by spin simulation.

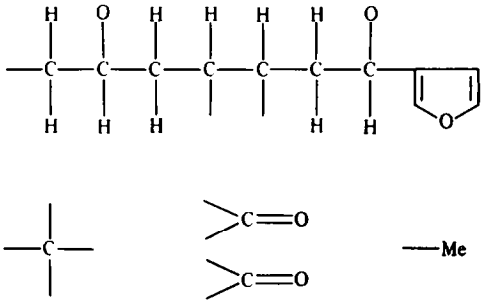


Fig. 1. Structural elements derived from spectroscopic data.

the latter dihedral angles, corresponding to the epimer shown in structure **5**. In the proton 2D-NOE spectra (270 MHz), the methyl protons showed interactions with H-2′, H-4′, H-4 (eq) (and/or H-4a) and H-10 (ax). This confirms the structure shown for deoxycollybolidol (**5**). The absolute configuration, however, still remains to be determined, and the choice of enantiomer shown in **5** is therefore arbitrary.

EXPERIMENTAL

General methods. Mps are corr. NMR spectra were recorded for $CDCl_3$ solns (~ 0.1 M). Chemical shifts are given in ppm downfield from $CHCl_3$ δ7.25 (^{1}H) and $CDCl_3$ δ77.17 (^{13}C). Assignments were confirmed by homo- and heteronuclear spin decoupling expts, 2D homo- and heteronuclear correlation spectroscopy, and 2D *J*-resolved spectroscopy. Relaxation time measurements in $CDCl_3$ (270 MHz) showed proton T_1 values 0.6–2.4 sec. In the 2D-NOE expts, mixing times of 0.6–1.0 sec were used. The spin simulation program included in the JEOL software package for an XL-100 instrument was used for determination of coupling constants and chemical shifts where second-order effects were present (see Tables 1–3). Silica gel 60 (0.040–0.063 mm, Merck) was used for CC. For TLC, Merck HPTLC plates (silica gel 60) were used. The spots were detected with anisidine reagent or by charring with H_2SO_4.

Extraction. Fruiting bodies of *Collybia peronata* were collected and frozen to −30° within 24 hr. The frozen material (100 g) was ground with dry ice in a blender and then mixed with H_2O and lyophilized. The dry powder (35.5 g) was stirred overnight with 200 ml $CHCl_3$–MeOH (2:1) and the procedure was repeated 2 × with fresh solvent. The combined extracts were concentrated and the residue (8.7 g) was mixed with 150 ml $CHCl_3$–MeOH–H_2O (8:4:3). The lower layer was concentrated and the residue (2.4 g) was applied to a column of silica gel, packed and eluted with EtOAc–MeOH–HOAc–H_2O (40:3:3:2). The appropriate fractions were pooled, concentrated and purified further on a second column. Elution with EtOAc gave pure material (49 mg). Crystallization from EtOAc–hexane gave material with mp 189–190°, $[\alpha]_D$ +21° (*c* 0.1; $CHCl_3$). Found: C, 65.2; H, 5.8. Calc. for $C_{15}H_{16}O_5$: C, 65.2; H, 5.8%. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1745 (δ-lactone), 1790 (γ-lactone). FABMS

m/z: 276 $[M]^+$. EIMS (probe, 70 eV) *m/z* (rel. int.): 276 $[M]^+$ (38), 179 (19), 110 (29), 98 (21), 94 (100).

Acknowledgements—We thank Professor B. Lindberg for his interest, Dr. S. Hoffman (Department of Analytical Chemistry, University of Stockholm) and Mr. A. Christakoupolous (Swedish National Institute of Environmental Medicine) for recording the EI and FAB mass spectra, respectively; Dr. Karin Leontein (National Bacteriological Laboratory) for generous help with recording of NMR spectra; Dr. Å. Stridh (Swedish Museum of Natural History) for fungal species examination; and the Swedish Council for Forestry and Agricultural Research for financial support.

REFERENCES

1. Bui, A.-M., Cavé, A., Janot, M.-M., Parello, J. and Potier, P. (1974) *Tetrahedron* **30**, 1327.
2. Pascard-Billy, C. (1972) *Acta Crystallogr. Sect. B* **28**, 331.

Phytochemistry, Vol. 25, No. 11, pp. 2663–2664, 1986.
Printed in Great Britain.

0031-9422/86 $3.00 + 0.00
Pergamon Journals Ltd.

ISODEHYDROLEUCODIN AND ANOTHER NOVEL *CIS*-LACTONIZED GUAIANOLIDE FROM *MONTANOA IMBRICATA*

FRED C. SEAMAN, NIKOLAUS H. FISCHER* and T. J. MABRY

Department of Botany, The University of Texas at Austin, Austin, TX 78713, U. S. A.; *Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803, U. S. A.

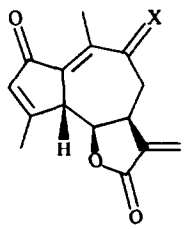
(*Received* 18 *March* 1986)

Key Word Index—*Montanoa imbricata*; Asteraceae; Heliantheae; sesquiterpene lactones; guaianolides.

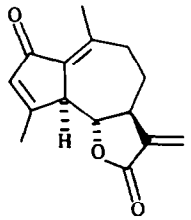
Abstract—*Montanoa imbricata* yielded two guaianolides, isodehydroleucodin, which is the C-5,C-6-isomer of dehydroleucodin, and 9-oxo-isodehydroleucodin.

The less polar member (**1**) of a pair of guaianolides isolated from *Montanoa imbricata* V. A. Funk displayed spectral data (^{1}H NMR, Table 1; UV; MS) that approximated those of dehydroleucodin (**3**) previously isolated from *Lidbeckia pectinata* Berg. (Asteraceae, Anthemideae) [1]. Differences in the ^{1}H NMR coupling constants and chemical shifts between **1** and **3** suggested that they were stereoisomers. Stereochemical arguments are presented to support the proposal of opposite orientations of H-5 and H-6 in **1** relative to **3**. The second *M. imbricata* guaianolide (**2**) differs from **1** only in the presence of a keto function at C-9 in **2**.

Assuming an α-orientation for H-7 as in all sesquiterpene lactones reported from higher plants [2], difference NOE studies were conducted to establish the relationship between H-5, H-6 and H-7. Irradiation of H-5, H-6 and H-7 indicated a strong NOE between H-6 and H-7. No effects were observed between either H-5 and H-6 or H-5 and H-7. The results indicated that H-6 shares an α-orientation with H-7 and that H-5 most likely has an opposite β-orientation. These findings are in agreement with the *J*-values of **1**: the large $J_{5,6}$ (11.3 Hz) and smaller $J_{6,7}$ (7.8 Hz) are consistent with an antiperiplanar relationship (*Ca* 180° dihedral angle) between H-5 and H-6



1 X = H,H
2 X = O



3