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MICROCYBIN: A NOVEL DIMERIC COUMARIN FROM *MICROCYBE MULTIFLORUS* AND *NEMATOLEPIS PHEBALIOIDES*

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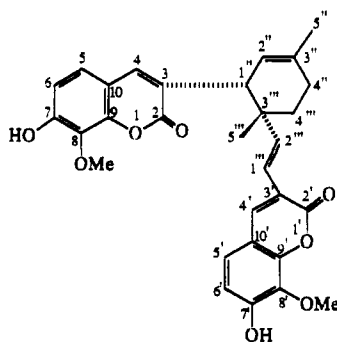
ABSTRACT.—A novel dimeric 3-prenylated coumarin **1** has been isolated from the aerial parts of two species of Rutaceae, *Microcybe multiflorus* and *Nematolepis phebalioides*. Compound **1** was identified, on the basis of spectral analysis, as (*rel*)-(*E*)-1-(7-hydroxy-8-methoxycoumarin-3-yl)-2-[1,4β-dimethyl-2α-(7-hydroxy-8-methoxycoumarin-3-yl)-cyclohex-1-en-4-yl]-ethene, to which we have assigned the trivial name microcybin. In addition, *N. phebalioides* yielded the furoquinolines evolitrine, skimmianine, flindersiamine, and halfordinine, and the pyranocoumarin seselin.

We have recently reported (1) the presence of a number of simple furocoumarins from the aerial parts of *Microcybe multiflorus* Turcz. (Rutaceae). The presence of these supports the argument (2) that the small genus *Microcybe* is closely allied to, or perhaps congeneric with, *Phebalium* sect. *Phebalium*, in which furocoumarins occur widely (3). In addition to furocoumarins, the sample of *M. multiflorus* yielded a new dimeric coumarin **1**, the structure of which is reported here. Parallel with the study of *M. multiflorus*, we undertook an investigation of *Nematolepis phebalioides* Turcz. (Rutaceae). *Nematolepis*, like *Microcybe*, is a small genus of questionable validity. *N. phebalioides* is suggested (2) to be allied to *Phebalium* sect. *Eriostemoides*, which is currently known to be a source of furocoumarins and 7-geranyloxy-coumarins (3).

The aerial parts of both species were

extracted sequentially with petroleum ether (bp 40–60°), EtOAc, and MeOH. Each extract was separately concentrated, and preliminary separation was achieved by use of vacuum liquid chromatography (vlc) over Si gel, eluting with solvents of increasing polarity. Compounds were isolated from the crude fractions obtained by means of further vlc, preparative tlc, or centrifugal preparative tlc. These procedures yielded several furocoumarins and triterpenes from *M. multiflorus* (1) and a number of known furoquinoline alkaloids and a pyranocoumarin from *N. phebalioides* (reported here). In addition, both extracts yielded a dimeric prenylated coumarin **1**, the structural elucidation of which is the subject of this paper.

The hreims of **1** indicated a molecu-



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lar formula of $C_{30}H_{28}O_8$ with a base peak at m/z 258 [$C_{15}H_{14}O_4$] $^+$. The ir spectrum suggested the presence of OH and C=O functional groups, and the strong maximum at 330 nm in the uv spectrum was indicative of a coumarin (4). The 1H -nmr spectrum (Table 1) revealed 28 protons which included two aromatic/olefinic singlets, trans-coupled olefinics, two sets of ortho-coupled aromatics, and two MeO singlets. The remaining resonances suggested the presence of CH-CH=C(Me) and CH₂-CH₂ spin systems and an isolated Me.

The comparability of the resonances

for the two coumarin systems suggested they were identical. The more deshielded doublet of each pair of ortho-coupled protons showed enhancements with the two aromatic/olefinic singlets in the NOESY spectrum. The chemical shift of the singlets suggested they must be placed at C-4, leaving the other two protons to occupy C-5 and C-6. The ^{13}C resonances attributable to the two coumarin nuclei were identified by direct 1J (HMQC) and long-range 2J and 3J (HMBC) coupling experiments, the latter confirming the positions of the three protons. Thus, 3J couplings from H-4 allowed identifica-

TABLE 1. 1H - and ^{13}C -nmr Chemical Shift Data and 2J and 3J 1H - ^{13}C Couplings (identified from HMBC spectrum) for Compound 1.^a

Position	δ_H	J	δ_C	2J	3J
2			163.2		
3			126.9		
4	7.86 s		142.4		163.2, 148.7, 124.2, 45.7
5	7.35 d	8.5	124.3		155.7, 148.4
6	7.08 d	8.5	115.0 ^b		135.7, 113.9
7			155.2		
8			135.7		
9			148.7		
10			113.9		
8-OMe	3.90 s		61.3		135.7
2'			161.1		
3'			121.9		
4'	7.83 s		138.1		161.1, 148.4, 124.2, 121.7
5'	7.14 d	8.6	124.2		155.7, 148.4
6'	7.07 d	8.6	114.9 ^b		135.6, 114.2
7'			155.7		
8'			135.6		
9'			148.4		
10'			114.2		
8'-OMe	3.88 s		61.3		135.6
1''	4.04 br s		45.7		
2''	5.40 br s		122.9		
3''			135.8		
4''	2.06 m		28.3		
	2.13 m				
5''	1.75 br s		26.0	135.8	122.9, 28.2
1'''	6.46 d	16.2	121.7		161.1, 148.4, 40.3
2'''	7.15 d	16.2	141.8		121.9
3'''			40.3		
4'''	1.61 m		30.8		
	1.87 m				
5'''	1.39 br s		23.8	40.3	141.8, 45.7, 30.8

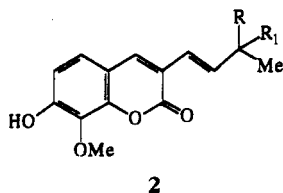
^aSpectra run in C₂D₂N₂.

^bAssignments may be interchanged.

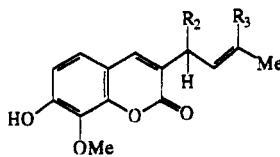
tion of C-2, C-5, and C-9; H-5 of C-4, C-7, and C-9; and H-6 of C-8 and C-10, while both MeO resonances showed long-range coupling to C-8 in each case. On this basis the dimer was concluded to contain two 7-hydroxy-8-methoxycoumarin nuclei that are substituted at C-3. The linking group contained 10 carbons which included a trans olefinic system.

Placement of the trans-olefin at C-3 of one of the coumarin units was established through 3J coupling of the δ 6.46 olefinic proton with C-2 and C-4 of the coumarin, and H-4 of the coumarin to one of the olefinic carbons. A further 3J coupling from the olefinic proton identified a quaternary carbon on the other side of the trans double bond. The Me, resonating at δ 1.39, was placed on this quaternary carbon as there was 3J coupling with the second olefinic carbon at δ_c 141.8 and 2J coupling with the quaternary carbon; this led to the partial structure **2**.

The H-4 proton of the second coumarin moiety exhibited a 3J coupling to the methine carbon at δ_c 45.7 (δ 4.04). This methine proton was part of the CH-CH=C(Me) spin system, which allowed assignment of a second structural fragment **3**. There remained only the problem of placing the CH₂-CH₂ system to complete the structure. That this was linked to the two quaternary Me-bearing sites was established by the observation of 3J interactions between the olefinic Me protons and the δ_c 28.2 methylene carbon and the protons of the other Me and the δ_c 30.8 methylene carbon. This permitted assignment of the full structure as **1** to which we have assigned the trivial name microcybin. Microcybin is a novel structure but is closely related to a number of other dimeric coumarins that have been isolated from the Rutaceae (4). The relative stereochemistry of the Me at C-3''' and the proton at H-1'' was established as *cis* by a NOESY experiment. As



2



3

with other such dimers it lacks optical activity and is, therefore, a racemic mixture, possibly formed through a Diels-Alder addition of 7-hydroxy-8-methoxy-3-(3-methyl-1,3-butadienyl)coumarin monomers.

In addition to microcybin, *N. phebalioides* yielded the pyranocoumarin seselin and the furoquinoline alkaloids evolitrine, skimmianine, flindersiamine, and halfordinine, all of which were identified by direct comparison with authentic samples. While *N. phebalioides* and *M. multiflorus* both yielded microcybin, *N. phebalioides* does not appear to produce the furocoumarins and 7-geranyloxycoumarins that typify species of *Phebalium* sect. *Eriostemoides* (3). Thus, while the chemical evidence for a close affinity between *M. multiflorus* and *Phebalium* sect. *Phebalium* is compelling, there is no clear support for a similar close affinity between *N. phebalioides* and *Phebalium* sect. *Eriostemoides* emerging from the metabolites so far isolated.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Uv, (Perkin-Elmer 552) in MeOH; ir, (Perkin-Elmer 781) KBr disks; specific rotations [α]_D, (Perkin-Elmer 241); nmr, Bruker AMX-400 (¹H at 400 MHz, ¹³C at 100.6 MHz); all pulse sequences use modified Bruker microprograms; eims AEI-MS902 double-focussing at 70 eV. Melting points (uncorrected), Reichert Hot Stage. Petroleum ether refers to the bp 60–80° fraction.

PLANT MATERIAL.—*N. phebalioides* samples (PERTH 01156659, 01155636) were collected from sandy roadside localities in the southern part of Western Australia and have been deposited at the Western Australian Herbarium, Perth. *M. multiflorus* samples (PERTH 01187694 and 01156721) were collected in similar localities and are likewise deposited at the Western Australian Herbarium, Perth.

EXTRACTION AND ISOLATION OF COMPOUNDS FROM *NEMATOLEPIS PHEBALIOIDES*.—The ground aerial parts (550 g) were extracted sequentially in a Soxhlet with petroleum ether, EtOAc, and MeOH. The concentrated petroleum ether extract was fractionated by vlc over Si gel, eluting with petroleum ether containing increasing amounts of EtOAc. Elution with 12% EtOAc yielded skimmianine (2 mg) and evolitrine (2.5 mg). Similar treatment of the EtOAc extract gave, in order of elution, halfordinine (2.5 mg), seselin (4 mg), microcybin [1] (7 mg), and flindersiamine (4 mg).

The isolation of microcybin [1] from *Microcybe multiflorus* (25 mg from 280 g of the aerial parts of the combined samples) has been detailed previously (1).

Seselin, skimmianine, evolitrine, flindersiamine, and halfordinine were all characterized by

direct comparison (uv, ir, nmr, eims) with material previously isolated and identified from other species of Rutaceae in the Strathclyde laboratory.

Microcybin [1].—Amorphous solid: $[\alpha]_D^{20}$ ($c=0.1$, CHCl_3); uv λ max 230, 245, 330 nm; ir ν max 3300, 1700, 1600, 1500, 1460, 1240, 1170, 1085, 970 cm^{-1} ; ^1H and ^{13}C nmr see Table 1; eims m/z (%) $[\text{M}]^+$ 516.1774 (calcd for $\text{C}_{30}\text{H}_{28}\text{O}_8$, 516.1784) (27), 311 (18), $[\text{M}^2]^+$ 258.5912 (0.7), 258 (100), 257 (29), 243 (45), 230 (20), 205 (17), 197 (20).

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