Synthesis and the Biological Evaluation of the Structural Units of Drummondin C

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Drummondin C (1) is an antibiotic isolated from a bioassay-directed fractionation of *Hypericum drummondii* (Grev.& Hook.)T.&G. It showed significant activity against the Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* and the acid-fast bacterium *Mycobacterium smegmatis*. Two structural units of drummondin C, the 8-acetyl-5,7-dihydroxy-2,2-dimethylchromene (6) and 5-acetyl-3-methyl-filicinic acid (9), were synthesized to determine the relative importance of the two substructure portions to the antibiotic activity of the compound. The low antimicrobial activity of 6 and 9 demonstrates the necessity of both units for the antibiotic activity of drummondin C.

KEY WORDS: antimicrobial activity; drummondin C; long-range COSY; chromene; filicinic acid; structural units.

INTRODUCTION

Hypericum drummondii, locally known as nits-and-lice, is a common weed in Mississippi. A new antibiotic drummondin C (1) was isolated recently from the hexane extract of the roots of the plant by a bioassay guided isolation procedure (1). Drummondin C showed significant activity against the Gram-positive bacteria *Staphylococcus aureus* with *Bacillus subtilis* and the acid-fast bacterium *Mycobacterium smegmatis*. Its structure is composed of a 3-acetyl-filicinic acid moiety connected to a 2,2-dimethylchromene moiety via a methylene bridge.

In an attempt to understand which feature of the drummondin C structure is responsible for the observed biological activity, its two structural units, 8-acetyl-5,7-dihydroxy-2,2-dimethylchromene (6) and 5-acetyl-3-methylfilicinic acid (9), were synthesized (Scheme I) and their antimicrobial activity was evaluated and compared with that of the parent compound drummondin C. The minimum inhibitory concentration (MIC) values toward a representative bacteria would allow us to judge whether structural fragments of drummondin C structure were sufficient to provide the observed antimicrobial activity.

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MATERIALS AND METHODS

All melting points were determined on a Fisher–Johns digital melting-point analyzer Model 355 and were not corrected. Infrared spectra (IR, KBr) were taken on a Perkin Elmer 281B spectrophotometer. Ultraviolet spectra (UV) were recorded on a Perkin Elmer, Lambda 3B UV/VIS spectrophotometer as methanol solutions. Low-resolution mass spectra were obtained on a Finnigan 3200 gas chromatograph/mass spectrophotometer operating in electron impact mode at 70 eV. Nuclear magnetic resonance spectra were recorded on a Varian VXR (300-MHz) spectrophotometer in deuterated acetone with TMS as internal reference. For the long-range COSY spectra, a delay time (D_3) of 0.3 sec was used in the pulse sequence.

Staphylococcus aureus (ATCC 6538), *Bacillus subtilis* (ATCC 6633), and *Mycobacterium smegmatis* (ATCC 607) used are deposited in the University of Mississippi, Department of Pharmacognosy, culture collection and were originally from the American Type Culture Collection (ATCC), Rockville, Maryland.

3-Methylbut-2-enyl bromide and 2,4,6-trihydroxyacetophenone were purchased from Aldrich Chemical Company. Thin-layer chromatography (tlc) analysis was performed by utilizing MN or Merck precoated plates. Column chromatography employed silica gel G_{60} (270–430 mesh ASTM).

2,4,6-Trihydroxy-3-isopentenylacetophenone (4)

3-Methylbut-2-enyl bromide (3; 6.2 g, 0.04 mol) was added dropwise over 1.5 hr to a stirred solution of 2,4,6-trihydroxyacetophenone 2 (7 g, 0.04 mol) in 10% aqueous potassium hydroxide (225 ml) and the mixture was stirred an additional 2 hr at room temperature under nitrogen. After this time the solution was poured into 10% hydrochloric acid and extracted with ether. The ether extracts were combined, dried, and evaporated and the residue was chromatographed on a silica gel column and eluted with chloroform to give initially biprenylated, triprenylated, and deacylated compounds. Further elution with chloroform/methanol/98% formic acid (98:1.75:0.25, v/v) furnished the desired monoprenylated acetophenone (4) and unreacted starting material (2.5 g). Compound 4 was obtained as light yellow needles from chloroform; m.p. 170–171°C[lit. 172°C (2)]; yield, 1.1 g (11%). (M^+)m/z 236, UV (MeOH)λ_{max} 290 nm. IR (KBr) ν max: 3420 (s, sharp), 3340 (s, broad), 2980 (w), 2920 (w), 1630 (s, broad), 1600 (s), 1450 (s) cm^{-1}. ^1H NMR (δ): 9.09, 9.48, 14.70 (1 H each, OH), 6.07 (1 H, s, H-5), 2.61 (3H, s, CH_3CO), 3.23 (2H, d, J = 7.2 Hz, H-1′), 5.22 (1 H, brt, J = 7.2, H-2′), 1.62, 1.74(3 H each, s, H-4′ and H-5′). ^{13}C NMR (δ): 1, 3-C (107.4, 105.0), 5-C (94.6), 2,4,6-C (160.4, 162.5, 164.6), 7-C (203.4), 8-C (32.5) 1′-C (21.6), 2′-C (123.7), 3′-C (130.4), 4′,5′-C (17.5, 25.6).

6-Acetyl-5,7-dihydroxy-2,2-dimethylchromene (5) and 8-Acetyl-5,7-dihydroxy-2,2-dimethylchromene (6)

DDQ (735 mg, 0.003 mol) was added to 4 (735 mg, 0.003 mol) in dry benzene and refluxed on a water bath for 30 min. The solution was filtered hot and evaporated to obtain a yellow residue. The residue was chromatographed on a silica

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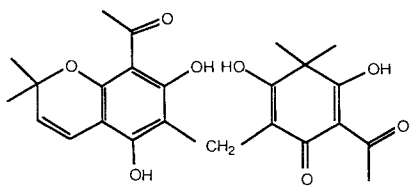

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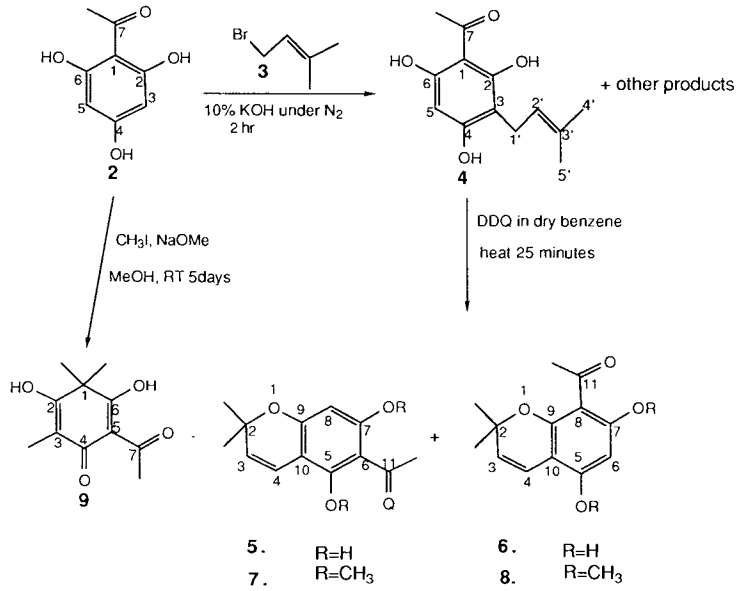

Scheme I. Synthesis of structural units **6** and **9** of drummondin C (**1**).

gel column eluting with toluene/ethylacetate/98% formic acid (98:1.75:0.25) to yield 6-acetyl-5,7-dihydroxy-2,2-dimethylchromene (**5**) and 8-acetyl-5,7-dihydroxy-2,2-dimethylchromene (**6**). Compound **5** was obtained as a yellow powder. Yield, 9.7 mg (1.3%). The substance decomposed at 200°C. (M^+) m/z 234, UV (MeOH)λ_{max} 292 nm. IR (KBr)ν_{max}: 3600–2500 (m, broad), 1630 (s), 1590 (s), 1440 (m) cm^{-1}. ^1H NMR (δ): 1.40(6H, s, 2,2-dimethyl), 5.53(1H, d, J = 9.9, H-3), 6.58(1H, d, J = 9.9, H-4), 9.99, 14.05 (1H each, s, 5 and 7 OH), 5.93 (1H, s, H-8), 2.62 (3H, s, CH_3CO). ^{13}C NMR (δ): 2-C (78.5), 3-C (126.1), 4-C (116.6), C-5,7,9 (161.0, 162.0, 162.8), 6,10-C (105.7, 102.6), 8-C (95.8), 11-C (204.2), 11-methyl (33.0).

Compound **6** was obtained as yellow needles from petether/chloroform (1:1, v/v); m.p., 136°C; lit., 135–136°C (3); yield, 71.8 mg (10%); (M^+) m/z 234, UV (MeOH)λ_{max} 278 nm. IR (KBr)ν_{max}: 3600–2500 (m, broad), 1640 (m), 1600 (s), 1430 (m) cm^{-1}. ^1H NMR (δ): 1.52 (6H, s, 2,2,dimethyl), 5.53 (1H, d, J = 9.9, H-3), 6.58 (1H, d, J = 9.9, H-4), 9.60 (1H, s, 5-OH), 5.96 (1H, s, H-6), 13.69 (1H, s, 7-OH), 2.63 (3H, s, CH_3CO). ^{13}C NMR (δ): 2-C (78.9), 3-C (125.4), 4-C (117.3), 5,7,9-C (157.9, 160.5, 166.8), 6-C (96.5), 8,10-C (102.9, 106.2), 11-C (203.7), 11-methyl (33.2).

Preparation of Methylethers 7 and 8

To the acylchromene (1 mol equiv) in dry acetone was added excess potassium carbonate (10× weight of the acylchromene) and dimethylsulfate (2 mol equiv) and the mixture refluxed in dry acetone (25 ml) under anhydrous conditions. After 10 hr the acetone was evaporated and the residue dissolved in water and extracted with ether. The ether layer was dried with sodium sulfate and evaporated to obtain a residue.

6-Acetyl-5,7-dimethoxy-2,2-dimethylchromene (**7**) was prepared from **5** (5.6 mg) as a light yellow gum (4.5 mg), which was purified by passing through a silica gel plug eluting with hexane/ethylacetate/formic acid (90:10:0.25, v/v) to obtain a white powder; m.p., 77°C [lit. 78–79°C (4)]; yield, 72%. (M^+) m/z 262, UV (MeOH)λ_{max} 278 nm. IR ($CHCl_3$) ν_{max}: 3100–2800 (m), 1685–1695 (m), 1607 (s), 1572, 1370 (m) cm^{-1}. ^1H NMR (δ): 1.42, (6H, s, 2,2-dimethyl), 5.66 (1H, d, J = 9.9, H-3), 6.5 (1H, d, J = 9.9, H-4), 3.72, 3.82 (3H each, s, 5.7-OCH_3), 6.29 (1H, s, H-8), 2.37 (3H, s, CH_3CO).

8-Acetyl-5,7-dimethoxy-2,2-dimethylchromene (**8**) was prepared from **6** (15 mg). The residue was crystallized from chloroform/hexane (1:1, v/v) to produce white needles; m.p., 106°C [lit., 108°C (3)]; yield, 10.6 mg (63%). (M^+) m/z 262, UV (MeOH) 278 nm. IR (KBr) ν_{max}: 2900–3000 (w, broad), 1706 (s), 1638 (s), 1583 (s), 1470 (m) cm^{-1}. ^1H NMR (δ): 1.38 (6H, s, 2,2-dimethyl), 5.55 (1H, d, J = 9.9, H-3), 6.55 (1H, d, J = 9.9, H-4), 6.30 (1H, s, H-6), 3.81, 3.88 (3H each, s, 5,7-OCH_3), 2.36 (3H, s, CH_3CO).

Preparation of 5-Acetyl-3-methylfilicinic Acid (9)

Compound **9** was obtained by reacting 2,4,6-trihydroxyacetophenone (**2**; 5 g, 0.03 mol) in absolute meth-

anol (20 ml), sodium methoxide (2 g sodium in methanol 35 ml), and methyl iodide (25 g, 0.18 mol) according to a previously described method (5). The desired product (**9**), which crystallized from the ether layer after concentration under vacuum, was obtained as white needles from petroleum ether/chloroform (1:1, v/v); m.p., 156–157°C [lit., 158°C (5)]; yield, 1.59 g (26%). (M^+) m/z 210, UV (MeOH)λ_{max} 345 nm. IR (KBr)ν_{max}: 3500–2500 (s, broad), 1656 (s), 1615–1560 (s, broad)cm^{-1}. ^1H NMR (δ): 1.37 (6H, s, 1,1-dimethyl), 1.88 (3H, s, 3-methyl), 2.51 (3H, s, CH_3CO), 8.4 (1H, s, 2-OH), 19.1 (1H, s, 6-OH). ^{13}C nmr (δ): 1-methyl (24.8); 1-C (49.1); 2,4,6-C (175.3, 190.4, 196.8); 3-methyl (7.2); 3,5-C (103.6, 106.5), 7-C (200.7), 7-methyl (29.3).

The Minimum Inhibitory Concentration (MIC) Determinations

The MIC values were determined using the twofold serial broth dilution technique previously described (6). All compounds were tested within the range of 0.20–100 μg/ml.

The MIC value was taken as the lowest concentration that inhibited growth of the microorganism after 24 or 48 hr of incubation. Tubes inoculated with *B. subtilis* were incubated at 30°C for 24 hr. Tubes inoculated with *S. aureus* and *M. smegmatis* were incubated at 37°C for 24 and 48 hr, respectively. Streptomycin sulfate was used as a positive control in the assay system.

RESULTS AND DISCUSSION

Compound **9** was prepared by alkylation of 2,4,6-trihydroxyacetophenone (**2**) with methyl iodide in sodium methoxide by a modification of the procedure described by Riedl and Risse (5).

Synthesis of **6** involved monoprenylation of 2,4,6-trihydroxyacetophenone (**2**) with prenylbromide (**3**) in 10% KOH in water, giving **4** as the major product in a mixture of diprenylated, triprenylated, and deacetylated products. Cyclization of **4** with DDQ afforded the two possible chromenes, **5** and **6**, which were separated by chromatography. The desired chromene (**6**) could not be distinguished readily from the 6-acetylated chromene (**5**) by conventional ^1H and ^{13}C NMR. Neither the Gibbs reagent (7) nor chemical shift changes of various protons in the acetylated derivatives (**8–11**) proved reliable enough to distinguish unequivocally between chromene **5** and chromene **6**. Since most natural chromenes possess at least a resorcinol, or a more complicated oxygenation pattern, making it difficult to differentiate possible isomeric structures, we undertook long-range COSY (12) experiments to evaluate their utility to solve this fairly common problem.

Methoxy groups show long-range proton–proton coupling to all adjacent *ortho* protons on an aromatic ring system (13). Consequently, one would expect the methyl derivatives of chromenes **5** and **6** to show differing long-range coupling patterns. Because this long-range coupling is quite small, it often appears only as a line broadening of the methoxy signals in a conventional proton magnetic resonance spectra, so that one cannot rigorously distinguish isomers. However, these very small couplings can easily be detected using "long-range COSY" spectral methods (12).

The methyl ethers **7** and **8** were prepared by reacting **5** and **6**, respectively, with dimethyl sulfate and potassium carbonate in acetone. Compounds **7**, *O*-methylevodionol, and **8**,

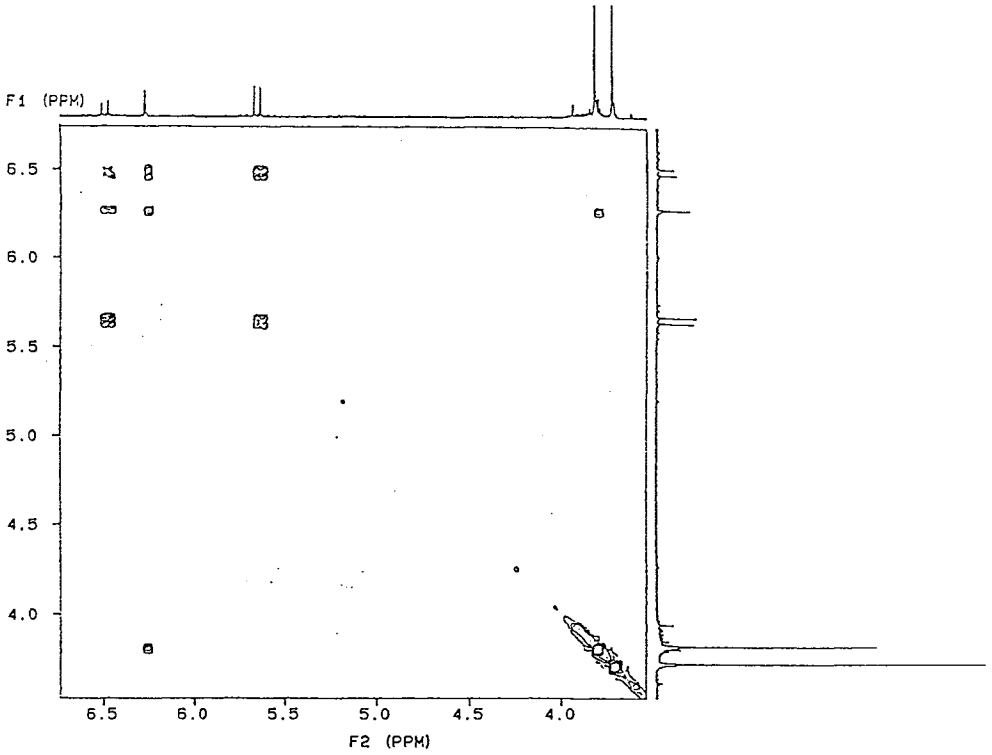

Fig. 1. Long-range COSY spectrum (δ3.5–6.5) of compound **7**.

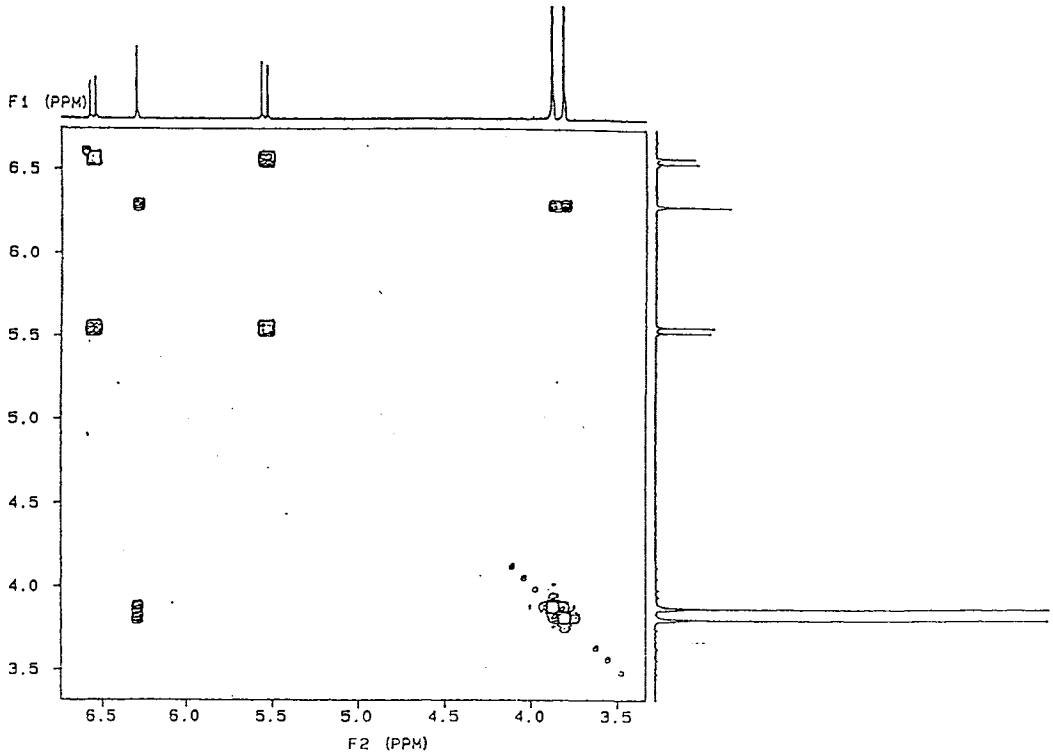

Fig. 2. Long-range COSY spectrum (δ3.5–6.5) of compound 8.

O-methylalloevodionol, have been reported as natural products isolated from *Acradenia franklinii* (Kippst) (4).

As can be seen in Fig 1, the H-8 signal at 6.29 ppm for 7 shows a cross peak with only one of the two methoxy peaks (the one at δ3.82), while the corresponding H-6 at 6.3 ppm for 8 shows (Fig. 2) cross peaks with both of the methoxy peaks (δ3.81 and δ3.88), thus confirming the assignment of the structures of 7 and 8 to the two isomeric cyclization products. This technique should prove quite useful to assign the structure of variously substituted chromenes. Interestingly it was also observed (Fig. 1) that for 7, H-8 (6.29 ppm) also showed an unusual long-range five-bond coupling to H-4 (6.5 ppm), a type of coupling which has been referred to as W coupling in similar chromene systems (8).

With both structural portions in hand, we could now evaluate their respective biological activities. The MIC values obtained for drummondin C and its two structural subunits **6** and **9** are reported in Table I. Drummondin C possesses activity comparable to streptomycin against the Gram-positive bacteria *S. aureus* and *B. subtilis* and shows moderate activity against the acid-fast bacterium *M. smegmatis*. Streptomycin is used as the positive control for the assay. The MIC values of **6** and **9** are much higher than that of the parent compound drummondin C. Clearly, both structural units are important for the activity.

Table I. Minimum Inhibitory Concentrations (μg/ml) of **1**, **6**, and **9**

Compound	*S. aureus*	*B. subtilis*	*M. smegmatis*
1	3.12	0.78	6.25
6	25.0	12.5	50.0
9	50.0	50.0	50.0
Streptomycin	6.25	0.78	1.56

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