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DITHIACYCLOHEXADIENES AND THIOPHENES OF *RUDBECKIA HIRTA*

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Abstract—The *Z*-isomers of a dithiacyclohexadiene and a thiophene polyine were isolated and identified from root cultures of *Rudbeckia hirta*, together with the known *E*-isomers. The structures were elucidated by spectroscopic methods. A complete ^{1}H NMR analysis of related dithiacyclohexadienes and thiophenes from *Chaenactis douglasii* is also given

INTRODUCTION

Previous investigations have shown the roots of *Rudbeckia hirta* L. (Asteraceae), a commonly grown ornamental, to contain thiophene- and 1,2-dithiacyclohexadiene-containing polyines [1]. As part of our continuing study of biologically active compounds from the Asteraceae and their synthesis in plant tissue culture, we have examined root culture extracts of *R. hirta*. Here we report the isolation and characterization of (*Z*)- and (*E*)-3-(1-propynyl)-6-(3,5-hexadien-1-ynyl)-1,2-dithiacyclohexa-3,5-diene (**1** and **3**) and their corresponding thiophenes (**2** and **4**) A complete ^{1}H NMR spectral analysis of the closely related antibiotics 3-(1-propynyl)-6-(5-hexen-3-yn-1-ynyl)-1,2-dithiacyclohexa-3,5-diene (**5**) and 2-(1-propynyl)-5-(5-hexen-3-yn-1-ynyl)-thiophene (**7**) from roots of *Chaenactis douglasii* (Hook.) H. & A is also described based on long-range ^{1}H–^{1}H couplings through six-bonds (^6J). Long-range ^{1}H–^{1}H couplings separated by more than three bonds have been extensively studied in saturated molecules and π systems [2–4] A knowledge of their magnitude can be of great assistance in the assessment of the structural influences on chemical shifts as well as on the stereochemistry.

RESULTS AND DISCUSSION

HPLC analysis of the petrol extract of *R. hirta* root cultures indicated the presence of four lipophilic compounds. In addition to the previously described **3** and **4** [1], their (*Z*)- isomers **1** and **2** were found. The major component of roots grown in liquid culture was **1** (63%), being present at three times the concentration of **3**. Thiophenes **2** and **4** were present in a similar ratio and together constituted 15% of total polyines.

The UV and mass spectral data of **1** and **2** corresponded closely to those of **3** and **4** Mass spectral analysis afforded molecular ions at m/z 230 for **1** and **3**, and m/z 198 for **2** and **4** Fragmentation patterns for each pair of compounds were almost identical, suggesting *E*/*Z*-isomers. GC-MS analysis using an SE-54 fused-silica capillary column resolved a mixture of **2** and **4** into two peaks ($R_t = 8.9$ and 8.5) with the same molecular ion, indicating **2** as the (*Z*)- and **4** as the (*E*)-isomer [5] This was consistent with the observation that **2** could be isomerized to **4** under UV.

Further evidence was provided by ^{1}H NMR analysis (Table 1). The spectrum of **1** was assigned on the basis of the observed three- and four-bond ^{1}H–^{1}H couplings (^3J and ^4J). A three-proton broad singlet at δ2.04 was attributed to the methyl group (for numbering scheme see Fig. 1). The thiophene protons H-8 and H-9, previously described as an AB quartet [1], were assigned to signals at δ6.67 and 6 50 (*br d*) respectively based on ^{1}H–^{1}H long-range coupling to the methyl group (see discussion of compounds **5** and **6** below). An ABX system corresponding to the vinylic protons was assigned to signals at δ5.52 (H-1), 5.40 (H-1′) and 6.85 (H-2). The splitting and broadening exhibited in these signals is as expected due to allylic coupling (^4J) with H-3 and H-4. Of the remaining signals, a broad doublet at δ5 72 with a ^3J value of 11 Hz typical of *cis*-olefins and a broad triplet at δ6.58 were assigned to H-4 and H-3, respectively. Both signals must also comprise a transoid ^4J for H-4 and H-2, and H-3 and H-1′ ($^4J_t = 1$ Hz), as well as a cisoid ^4J between H-3 and H-1 ($^4J_c < 1$ Hz), similar to those observed in substituted 1,3-butadienes [6]. These couplings, in addition to a ^3J value between H-2 and H-3 of 10.5 Hz, suggest that the conformation of the dienyl residue in solution is primarily *s-trans* (Fig 1)

Splitting patterns for thiophenes **2** and **4** were similar to those recorded for **1** Coupling constants (^3J) between H-3 and H-4 of 11 and 16 Hz, respectively, identified **1** and **2** as the (*Z*)- and **3** and **4** as the (*E*)-isomers. Allylic coupling in compounds **2** and **4** indicate that these are also *s-trans*

Although **5** has been described previously, its ^{1}H NMR spectrum in tetrachloromethane was only partially assigned [1] An ABX pattern was observed for the three vinyl protons, a singlet at δ2 05 for the methyl group, and an AB-quartet at δ6 60, corresponding to the unidentified H-8 and H-9 signals.

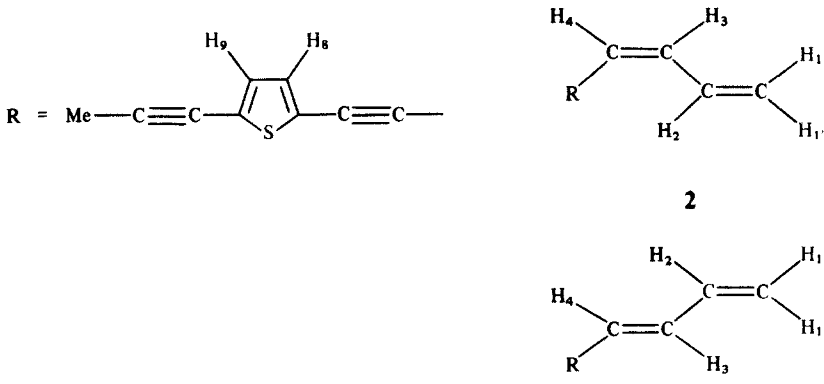
The ambiguity of assigning ring-proton signals in natural products with substituted thiophene and 1,2-dithiacyclohexadiene rings can be removed in some instances when long-range coupling is observed. Close examination of the 300 and 400 MHz ^{1}H NMR spectra of **5** in CD_3CN (Table 1) revealed that of these two un-

Table 1 ^{1}H NMR spectral data of compounds **1**, **2**, **4–8** (400 MHz, CD_3CN, TMS as int standard)

H	**1**	**2**	**4**	**5**	**6**	**7***	**8***
1	5.52 *ddd*	5.49 *ddd*	5.41 *ddd*	5.90 *dd*	5.78 *dd*	5.77 *dd*	5.79 *dd*
1′	5.40 *ddd*	5.37 *ddd*	5.26 *ddd*	5.78 *dd*	5.90 *dd*	5.67 *dd*	5.68 *dd*
2	6.85 *dddd*	6.90 *dddd*	6.49 *dddd*	6.01 *dd*	6.03 *dd*	6.07 *dd*	6.10 *dd*
3	6.58 *br t*	6.54 *br t*	6.70 *br dd*	—	—	—	
4	5.72 *br d*	5.73 *br d*	5.93 *br d*	—		—	—
6	—			—	—	6.68 *d*	7.15 *d*
7	—	—	—	—	—	6.78 *d*	7.26 *d*
8	6.67 *d*	7.11 *d*	7.07 *d*	6.79 *d*	7.27 *d*	—	—
9	6.50 *br d*	7.03 *br d*	7.01 *br d*	6.62 *dd*	7.05 *dd*		—
13 (Me)	2.04 *br s*	2.12 *br s*	2.06 *br s*	2.07 *s*	2.10 *br s*	2.05 *br s*	2.04 *s*

J[Hz] 1,1′ = 2, 1′,2 = 11, 1,2 = 17.5, compound **1** 1,3 < 1, 1′,3 = 1, 2,3 = 10.5, 2,4 = 1, 3,4 = 11, 8,9 = 7, 13,9 = 0.6, compound **2** 1,3 < 1, 1′,3 = 1, 2,3 = 10.5, 2,4 = 1, 3,4 = 11, 8,9 = 4, 13.9 = 0.3, compound **4** 1,3 = 1, 1′,3 < 1, 2,3 = 10.5, 2,4 < 1, 3,4 = 16, 8,9 = 4, 13,9 = 0.3, compound **5** 8,9 = 7, 13,9 = 0.6, compound **6** 8,9 = 4, 13,9 = 0.3, compound **7** 8,9 = 7, compound **8** 8,9 = 4

*Assignments for H-6 and H-7 obtained from ref [7]



4

Fig 1 Compounds **2** and **4** showing *s-trans*-conformation and the numbering scheme used for all compounds

assigned signals, H-8 and H-9, the latter appears not as a doublet but as a doublet of doublets in which the second coupling constant is very small, $^6J < 1$ Hz. As shown by double resonance experiments, the three-proton broad singlet at δ2.07 corresponding to the methyl resonance was found to be long-range coupled to the proton at C-9 ($^6J = 0.6$ Hz), identifying this signal (δ6.62) as H-9. Therefore the remaining signal at δ6.79 must be that of H-8 Because long-range coupling is sensitive to stereochemical influences, the ^{1}H NMR spectrum of the thiophene derivative **7** provides a smaller value for the analogous coupling between H-9 and the methyl group, $^6J = 0.3$ Hz, compared to the six-membered ring in **5**.

In both cases long-range ^{1}H–^{1}H coupling measurements were a convenient aid in the assignment of the ring-proton signals, furnishing information that had not previously been accessible [7] Increased conjugation of the corresponding 1-propynyl residue of thiophene or 1,2-dithiacyclohexadiene rings of related compounds in the form of a triple bond (**7** and **8**) did not produce any appreciable splitting in the methyl- or ring-proton signals. Preliminary results indicate that **1** exhibits antibacterial and antifungal activity comparable to that of **5** [8].

EXPERIMENTAL

Tissue cultures R hirta seeds were obtained from Richters & Sons Ltd in Goodwood, Ontario After surface sterilization with 5% hypochlorite soln, the seeds were germinated on moistened filter paper The emerging rootlets were excised, transferred to solid Schenk and Hildebrandt [9] medium (SH), and subsequently to liquid SH medium containing 0.5 mg/l naphthalene-acetic acid Cultures were maintained in 250 ml Erlenmeyer flasks on a rotary shaker (100 rpm) at 25° in the dark, and subcultured or harvested after 3 weeks *C douglasii* roots were cultured as described elsewhere [10]

Extraction of polyines. Root cultures were blotted dry, homogenized, filtered, and extracted with MeOH The extract was partitioned with petrol, concd and chromatographed on a silica gel column (Merck, 70–230 mesh), using petrol as solvent Yields were 0.44 mg/g extracted dry wt (EDW) total **1** and **3**, and 0.07 mg/g EDW total **2** and **4** All procedures were performed in dim light to prevent photodegradation of the compounds. Samples were stored in petrol at −20° or analysed immediately.

Analysis. Extracts for HPLC analysis were taken to dryness and resuspended in HPLC grade MeOH. Samples (20 μl) were injected on a Varian 5000 Liquid Chromatograph equipped with a Varian MCH-10 reverse-phase column (4 × 300 mm) using MeCN–H_2O (6:4) at a flow rate of 1.5 ml/min. Retention times and relative areas were calculated on a Spectra-Physics 4100 Computing Integrator using 2-phenyl-5-(4-hydroxy-1-butynyl)-thiophene as an int. standard. Compounds **1**–**4** eluted at R_t 24.6, 23.3, 19.9, and 16.8 min, respectively. UV spectra were obtained in petrol from HPLC-purified samples. Compound **1**: UV $\lambda_{\max}^{\text{petrol}}$ nm 482, 350, 257, GC-MS *m/z* (rel int.) 230 $[M]^+$ (54), 197 $[M\text{-}SH]^+$ (38), 165 $[M-SSH]^+$ (100). Compound **2**: UV $\lambda_{\max}^{\text{petrol}}$ nm 339, 248, GC-MS *m/z* (rel int): 198 $[M]^+$ (100), 165 $[M-SH]^+$ (27).

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