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SHORT REPORTS

POLYACETYLENES FROM *PTEROCAULON* SPECIES

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Key Word Index—*Pterocaulon allopecuroides, P. balansae, P. lanatum, P. rugosum*; Compositae–Inuleae; pentynes and thiophene acetylenes.

Abstract—Analysis of ethereal extracts from the roots of *Pterocaulon allopecuroides, P. balansae, P. lanatum* and *P. rugosum* gave two pentynes, of which one was new (tridec-1,2-dimethoxy-3,5,7,9,11-pentyne), and six yne-thiophene-diyne derivatives.

INTRODUCTION

The presence of coumarins in all *Pterocaulon* species investigated up to now [1–4], is of infragenerical chemotaxonomic significance. The occurrence of thiophene acetylenes in the roots of *P. virgatum* [4] confirmed that the genus belongs to the tribe Inuleae. However, its relationship with *Sphaeranthus* or *Pluchea*, two genera belonging to Plucheineae subtribe, remains to be established although it has been reported that both produce thiophene acetylenes [5].

We now report on the polyacetylenes of four further *Pterocaulon* species (*P. allopecuroides, P. balansae, P. lanatum* and *P. rugosum*). From the roots of these plants, we isolated and identified the polyacetylenes **1–8**, of which **2** is a new compound. We also detected the presence of a compound with a diene-triyne chromophore.

In a previous paper [2], we mentioned how the species *P. allopecuroides* and *P. balansae* are morphologically similar, making it difficult to distinguish between them. Our results clearly indicate their phytochemical difference.

RESULTS AND DISCUSSION

The occurrence and relative quantities of polyacetylenes (**1–8**) in the four species are given on Table 1. The compounds were isolated either by PLC (continuous elution, low temperature [6]) or by using a Chromatotron [7]. Their molecular structures were proposed on the basis of spectral data (UV, IR, ^{1}H NMR and MS) and the preparation of some derivatives. All spectral data were compared with those of the literature and confirmed the proposed structures.

The pentayne-ene **1**, occurs in all Inuleae species examined so far [5], but the new 1,2-dimethoxy derivative **2** is found only in *P. balansae*. Its UV spectrum is superimposable on those of 1,2-diol and 1.2-diacetate analogues [8]. The ^{1}H NMR data are also in agreement with structure **2**. We could not determine its optical rotation on account of the tiny amount of material which was available and its high unstability. However, the formation of **2** as an artifact was ruled out because no MeOH was used in the isolation process.

Compounds **3** and **6** co-occur in the four species examined. Compound **3** was first isolated from a *Schkuhria* species [9], and its structure was established unequivocally. In fact, by inserting another thiophene ring in **3** by the method of Schulte *et al.* [10], we obtained a product the ^{1}H NMR spectrum (80 MHz, CCl_4) of which showed the signals [δ2.04 (*s*, 3H), 5.08 (*d*, 1H, $J = 10$ Hz) and 5.42 (*d*, 1H, $J = 17$ Hz), respectively corresponding to methyl and terminal methylene protons and 6.50–7.00 (5H, H_{A-E})] expected for compound **9** [11]. The mass spectrum contained $[M]^+$ (*m/z*) 230, (100%) and $[M + 2]^+$ (10%) ions. The UV ($\lambda_{max}^{Et_2O}$ 362 nm) and IR [$\nu_{max}^{CCl_4}$ 2210 (–C≡C–, *ω*) and 1600 (–CH=CH$_2$, *s*) cm^{-1}] spectral data were also in agreement with those previously published [11].

Compound **6** gave the expected UV spectrum [5]. Apparently it was first isolated from an *Echinops* [12] but the published ^{1}H NMR data differs from ours. Later on, it was synthesized [13] and its spectral data corrected.

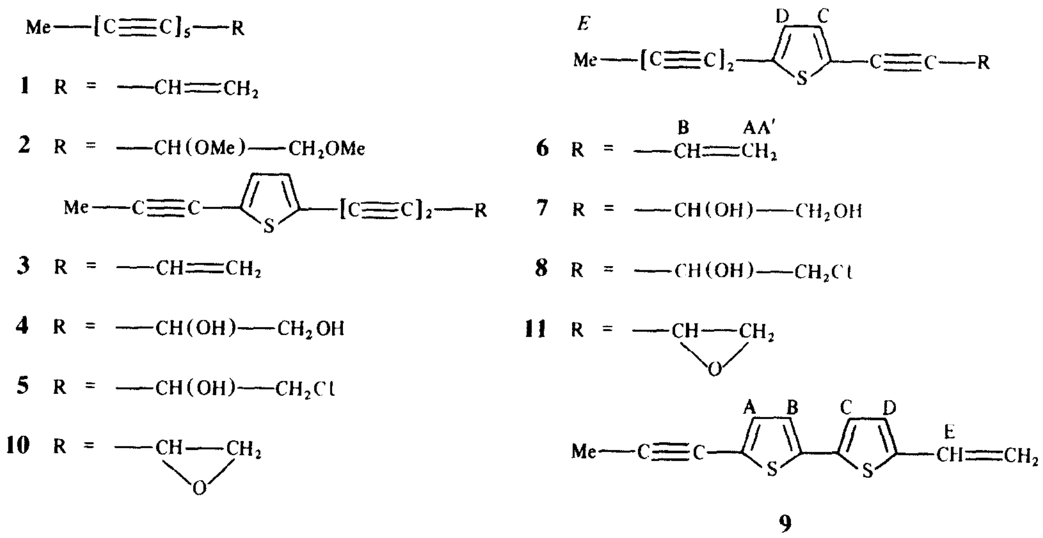
The chlorohydrines **5** and **8** occur in almost all investigated *Pterocaulon* species, except *P. allopecuroides*. Their UV spectra are almost superimposable and agree with those in the literature [4]. The corresponding ^{1}H NMR data however showed some discrepancies with the published data [4, 14] leading us to prepare the epoxides (**10** and **11**) by the method of Bohlmann *et al.* [15]. Structure **11** was proved by synthesis [13] and its ^{1}H NMR data coincide with ours.

Analysis of the ^{1}H NMR spectral data of the natural isomeric thiophene derivatives pairs isolated in this study led us to the conclusion that compounds having the propynylthiophene system show a greater chemical shift difference between thiophene protons than those having the pentadienylthiophene system. Thus for the epoxides

H	4	5*	6	8*	10
A	3.79*d*	3.62*dd*	6.00*m*	3.62*dd*	2.85*d*
A′		3.65*dd*		3.65*dd*	
B	4.65*m*	4.71*m*	5.5*m*	4.71*m*	3.36*d*
C	6.90*d*	6.87*d*	6.88*d*	6.97*d*	6.86*d*
D	7.18*d*	7.05*d*	7.07*d*	7.05*d*	7.07*d*
E	2.09*s*	2.08*s*	2.06*s*	2.04*s*	2.05*s*

J (Hz) = AA′ = 5.0; A′B = 5.5; CD = 4.0

*In $CDCl_3$



10 and **11**, we observed shift differences of 0.21 and 0.04 ppm for the thiophene protons, for **4** and **7** 0.28 and 0.14 ppm and for the chlorohydrines **5** and **8** 0.18 and 0.08 ppm. The signal values related to **5** and **8** in the table published by Bohlmann *et al.* [4] need to be interchanged.

The pair of compounds represented by **4** and **7** occurs only in *P. allopecuroides*. Their UV spectra are coincident. The former (**4**), was apparently isolated from a *Platycarpha* species [15], but its UV data are different from ours. Its structure was elucidated through ^{1}H NMR spectral analysis of the corresponding oxidation product. Its isomer **7** has been isolated from the genus *Echinops* [12]. Later on the structure was proved by synthesis [13]. In fact our spectral data, together with those of its epoxide derivative (**11**), agree with the literature [12, 13].

We detected the presence of one more polyacetylene common to *P. balansae* and *P. rugosum*. It occurs in very tiny amounts in the chromatographic fractions of the extracts containing **8** as the main compound. Its presence in the mixtures was shown by the presence of a sharp IR absorption band at 3300 cm^{-1} (ν H–C≡C) and a ^{1}H NMR singlet at δ2.50 (H–C≡C–C≡C) [13].

EXPERIMENTAL

General. IR:CCl_4; UV:Et_2O; MS (EI): 70 eV. Fresh roots were cut and percolated with Et_2O at 10° for *ca* 10 days (3 successive

Table 1. Distribution and abundance of the polyacetylenes in *Pterocaulon* species

	Compounds (mg/kg of fresh roots)							
Species	1	2	3	4	5	6	7	8
P. allopecuroides (Lam) DC.	83	—	56	28	—	13	24	—
P. balansae Chodat.	67	7	51	—	5	7	—	8
P. lanatum O. Kuntze	28	—	43	—	3	6	—	4
P. rugosum (Vahl.) Malme	62	—	38	—	8	5	—	18

extr). The extracts were dried (Na_2SO_4), the solvent removed in a rotary evaporator in the dark, at room temp. and the residue subjected to silica gel CC using petrol–Et_2O mixtures containing increasing amounts of Et_2O (assayed by UV). The fractions containing polyacetylenes were successively rechromatographed on a Chromatotron (gradient elution petrol–Et_2O; 5 ml/min) and by PLC (continuous elution) at 0°, in the dark, providing pure compounds.

Pterocaulon allopecuroides (Lam) DC. Plants were collected in February 1986 in Campinas (SP-Brasil). A voucher specimen is deposited in the Herbarium under No. 2706 (UEC). Fresh roots (690 g) provided **1** (58 mg), **3** (39 mg), **4** (20 mg), **6** (9 mg) and **7** (17 mg).

P. balansae Chodat. Plants were collected in August 1986 in Campinas. A voucher specimen is deposited in the Herbarium under No. 2709 (UEC). Fresh roots (1800 g) afforded **1** (120 mg), **2** (13 mg), **3** (92 mg), **5** (9 mg), **6** (13 mg) and **8** (14 mg).

P. lanatum O. Kuntze. Plants were collected in March 1987 in Americana (SP). A voucher specimen is deposited in the Herbarium under No. 2710 (UEC). Fresh roots (3700 g) provided **1** (104 mg), **3** (160 mg), **5** (11 mg), **6** (22 mg) and **8** (15 mg).

P. rugosum (Vahl.) Malme. Plants were collected in Ibitinga, near S. Carlos (SP). A voucher specimen is deposited in Herbarium under No. 25192 (UEC). Fresh roots (1300 g) provided **1** (81 mg), **3** (50 mg), **5** (10 mg), **6** (7 mg) and **8** (23 mg).

Tridec-1,2-*dimethoxy*-3,5,7,9,11-*pentyne* (**2**). Very unstable liquid; UV $\lambda_{max}^{Et_2O}$ nm (ε): 237.5 (104 000). 250 (226 000) and 264 (334 000); ^1H NMR (80 MHz, CCl_4): δ2.02 (*s*, 3H), 3.22–3.31 (*m*, 3H), 3.70 (*s*, 6H).

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A DIHYDROXYCYCLOPENTADIENONE AND OTHER CONSTITUENTS FROM THE SEEDS OF *TRIFOLIUM REPENS*

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Key Word Index—*Trifolium repens*; Leguminosae; white clover; fatty acids; sterols; triterpene; flavonols; (2*R*,3*R*)-butanediol; 3-hydroxy-2-methyl-4-pyrone; 2,3-dihydroxy-2,4-cyclopentadien-1-one.

Abstract—A new natural substance, 2,3-dihydroxy-2,4-cyclopentadien-1-one, was isolated from the seeds of white clover along with many known compounds.

INTRODUCTION

There are a number of papers on the constituents of the seeds of *Trifolium repens* L. [1–4], in some of which it is reported that myricetin and some condensed tannins are toxic to *Rhizobium* bacteria [2, 4]. In the course of our studies on biologically active natural products, we re-examined the constituents of the seeds. Although no new active compounds were obtained, a new natural substance, 2,3-dihydroxy-2,4-cyclopentadien-1-one (**1**), was isolated along with many known compounds, of which (2*R*,3*R*)-butanediol (**2**) and 3-hydroxy-2-methyl-4-pyrone were isolated for the first time from clover.