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PIPTOCARPHOL ESTERS AND OTHER CONSTITUENTS FROM *VERNONIA COGNATA*

ALICIA BARDON, CÉSAR A. N. CATALAN, ALICIA B. GUTIÉRREZ* and WERNER HERZ*

Instituto de Química Orgánica, Facultad de Bioquímica y Farmacia, Universidad Nacional de Tucumán, Ayacucho 491, 4000 S. M. de Tucumán, Argentina; *Department of Chemistry, The Florida State University Tallahassee, Florida 32306, U.S.A.

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Key Word Index—*Vernonia cognata*; Vernonieae; Compositae; glaucolides; piptocarphol esters; hirsutinolides; sesquiterpene lactones.

Abstract—Chemical investigation of *Vernonia cognata* resulted in isolation of glaucolide B, two piptocarphol diesters and several other well-known plant constitutents. A method for distinguishing between C-1 epimeric hirsutinolides is proposed.

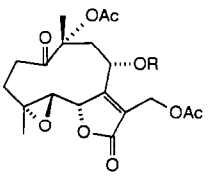
INTRODUCTION

In continuation of our work on Argentine *Vernonia* species [1–3], we have studied *V. cognata* Less. A collection of this species from an undisclosed location in Brazil [4] had given the hirsutinolide or piptocarphol diester **2a** which had previously been found in *V. scorpioides* and *saltensis* [5], *Stokesia laevis* [6] and later in *V. mollissima* [2] although in refs [4–6] the C-8 and C-10 stereochemistry and in ref. [2] the C-8 stereochemistry were misrepresented (for corrections see [3, 7]). Our own collection from Entre Rios Province, Argentina, furnished as the main sesquiterpene lactone constituent glaucolide B (**1a**) [8], considerably smaller quantities of **2a** and a small amount of what we believe to be a C-1 epimer of **2a** which appears to be new. In addition, various triterpenes and sterols were isolated.

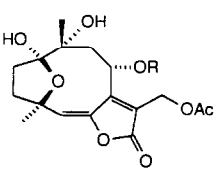
The literature on **2a** and similar substances is somewhat confusing. A survey of the ^{1}H NMR spectra of such hemiacetals and the corresponding 1-alkoxy derivatives from *Vernonia* and related species (for references see [3, 7]) reveals the existence of two distinct groups–group A with $J_{8,9a}$ and $J_{8,9b}$ differing widely in magnitude (1–3 and 8–11 Hz, respectively) and group B in which these two coupling constants are equal and relatively small (~3 Hz). In members of group A the H-8 resonance is unusually far downfield (in $CDCl_3$ δ6.3–6.5), whereas the H-8 signal of group B is found in the vicinity of δ 5.5. The relative configuration of members of group A which *inter alia* includes **2a** and other piptocarphol derivatives is as depicted in the formula because (a) compound **2b** has been correlated with glaucolide A (**1b**) of known stereochemistry, thus fixing the stereochemistry at C-4, C-8 and C-10 and (b) in **2a** and its analogues a strong intramolecular hydrogen bond exists between the two hydroxyl groups on C-1 and C-10 [9]. This can occur only if both hydroxyl groups are *cis*, i.e. if the configuration of **2a** and its analogues is 1*S**,4*R**,8*S**,10*R**. The paramagnetic shift of H-8 in this group thus appears to be due to deshielding by the 7,11-double bond [7] rather than to proximity to the ether oxygen [9] and the Dreiding model of these compounds is imprecise in predicting two small coupling constants rather than the one large coupling constant actually found for H-8 in members of this group.

Group B includes *inter alia* compounds formerly assigned structures **12** of [5], **3**, **4**, **5** and **6** of [6], **3** and **4** of [10] and **1** and **2** of [11] as well as our new very minor constituent of *V. cognata* (see Table 1 which also shows that the chemical shift is not affected significantly by changing the solvent from $CDCl_3$ to C_6D_6).

What is the stereochemistry of compounds belonging to group B? A change in C-10 stereochemistry should not affect the relationship of H-8 to the 7,11-double bond which presumably accounts for the deshielding of H-8 in members of group A. This is demonstrated by the X-ray analysis of compound **8b** of [7] which belongs to group A, but lacks the C-10 hydroxyl and has the C-10 methyl



1a R=Ac
b R=MeAcr



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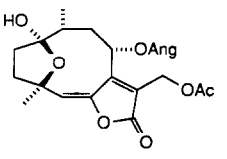
b R=MeAcr



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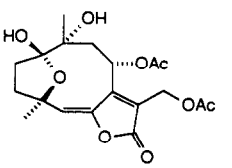

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Table 1. ^{1}H NMR spectral data of compound 4 (270 MHz)

H	$CDCl_3$	C_6D_6
2a, b	1.95–2.85	1.4–1.7
3a, b		2.1–2.4
5	6.04*s*	5.59*s*
8	5.54 *t* (3.5)	5.46 *dd* (4,3.5)
9a	2.61 *dd* (15,3.5)	3.47 *dd* (14,3.5)
9b	2.22 *dd* (15,3.5)	2.09 *dd* (4)
13a	5.06 *d* (13)	4.96 *d*
13b	4.86 *d* (13)	4.82 *d*
14*	1.24 *s*	1.02
15*	1.58 *s*	1.14
Ac*	2.11	1.57
	2.10	1.15

*Intensity three protons.

group *alpha*. A change in configuration at C-8, with the ester group β-orientated, would bring H-8, no longer deshielded by the double bond, closer to the ether oxygen with the effect on the H-8 difficult to predict in a conformation that accounts for the observed coupling constants. However, we give preference to a change in configuration at C-1, i.e. 1*R**. In the relatively rigid model of such a compound a β-orientated H-8 would no longer be deshielded by the 7,11-double bond and the observed coupling constants would be rationalized as well. Moreover, the isolation of C-1 epimers from the same plant extract, either as naturally occurring substances or as artefacts, can be justified easily on biogenetic or chemical grounds, more easily so than if the two series were epimeric at C-8.

EXPERIMENTAL

General. For separation of mixtures Waters HPLC equipment (M45 pump, U6K injector with 2 ml loop and R-401 differential refractomer) was used. The column employed was an Altex Ultrasphere ODS column (5 mm, 10 mm i. d. × 25 cm). R_fs were measured from the solvent peak.

Plant material. Aerial parts of *V. cognata* Less. were collected at the end of the flowering stage (seed formation) by Mr J. M. Retamal in 'Los Arenales', Paraná, Entre Ríos Province, Argentina. Voucher specimens (nos 260 and 310) were deposited at IPNAYS, Facultad de Ingeniera Química, Universidad Nacional del Litoral, Argentina.

Extraction of V. cognata. Fruits and leaves (218 g) of *V. cognata* were extracted with 2 × 4 l of $CHCl_3$ at room temp. for 7 days to give 15 g of extract which was suspended in 130 ml EtOH at 50–55°, dild with 95 ml H_2O and extracted successively with hexane (3 × 300 ml) and $CHCl_3$ (3 × 300 ml). Evaporation of the hexane extract gave 10.5 g residue. A portion of residue (5 g) was dissolved in hexane–EtOAc (7:3, 100 ml), decolorized with charcoal, filtered and saponified with dil. KOH. The unsaponifiable material was chromatographed over silica gel using hexane with increasing amounts of Et_2O (from 10–30%). This gave 300 mg pentacyclic triterpenes and 14 mg sterols. Reversed-phase HPLC of the triterpene fraction (MeOH), flow rate 2.4 ml/min) gave three peaks. The first was identified as lupeol, the second was a mixture of β-amyrin and taraxasterol (GC analysis) and the third a mixture of α-amyrin and ψ-taraxasterol. (GC analysis). The ratio of α-amyrin:β-amyrin:taraxasterol:ψ-taraxasterol:lupeol was 3:5:7:3:6. Triterpenes were identified by co-injection with authentic samples in HPLC, GC and by MS. Separation of the sterol fraction by reversed-phase HPLC afforded cholesterol, isofucosterol, stigmasterol and sitosterol (ratio 1:1:25:40).

Evaporation of the $CHCl_3$ extract yielded a residue (2 g) which showed one main spot on TLC and was purified by CC (silica gel, $CHCl_3$ with increasing amounts of Et_2O, 0–30%), 63 fractions being collected. Fr. 16–28 gave 1.3 g of the main constituent further purified by PPC (C_6H_6–Me_2CO 4:1) and identified as glaucolide B (**1a**) by comparison (^{1}H NMR, IR, mp, mmp) with material isolated from *V. fulta* [1]. Frs 29–30 purified by HPLC (MeOH–H_2O 4:3, flow rate 2 ml/min) gave 1.5 mg **4**. Preparative TLC (C_6H_6–Me_2CO 4:1) of frs 31–33 gave 30 mg **2a** whose spectral properties agreed with **2a** from *V. mollissima* [2] and those reported in the literature [4–6]. Purification of frs 34–43 by HPLC (MeOH–H_2O 4:3) gave an additional 17.6 mg **2a** and 12.7 mg impure **1a**. Frs 44–63 after HPLC (MeOH–H_2O 4:3) also furnished 14.7 mg **2a**.

Extraction of the roots of *V. cognata* (780 g) with $CHCl_3$ gave 10 g of residue which when chromatographed over silica gel ($CHCl_3$ and $CHCl_3$–EtOAc mixtures from 0–50%) afforded a large amount of lipids which were not investigated further. Fractions eluted with $CHCl_3$–EtOAc (1:1) gave stigmasterol.

(1*R**, 4*R**, 8*S**, 10*R**)-8,13-*Diacetoxy*-1,4-*epoxy*-10-*hydroxy*-*germacra*-5E,7(11)-*dien*-6,12-*olide* (**4**) was a gum, ^{1}H NMR: Table 1; MS *m/z* (rel. int.): 336 $[M^+ - AcOH]^+$ (1.5), 318 (1.7), 276 (1.4), 258 (0.7); MS PCI *m/z* (rel. int.): 397 $[M+1]^+$ (1.7), 382 (0.8), 380 (9.7), 379 (46.9), 337 (100), 319 (57.9), 277 (52.4), 259 (96); 241 (43.5).

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