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LOCALIZATION OF THE ACYL GROUPS IN PROAZULENE GUAIANOLIDES FROM *THAPSIA TRANSTAGANA* AND *THAPSIA GARGANICA*

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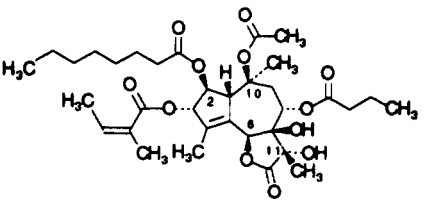
ABSTRACT.—A new esterified oxygenated guaianolide **3** possessing the terpenoid skeleton of the proazulene **2** previously isolated from *Thapsia garganica* was isolated from *Thapsia transtagana*. The locations of the acyl groups in **2** and **3** were established by partial hydrolysis and by spectroscopic means.

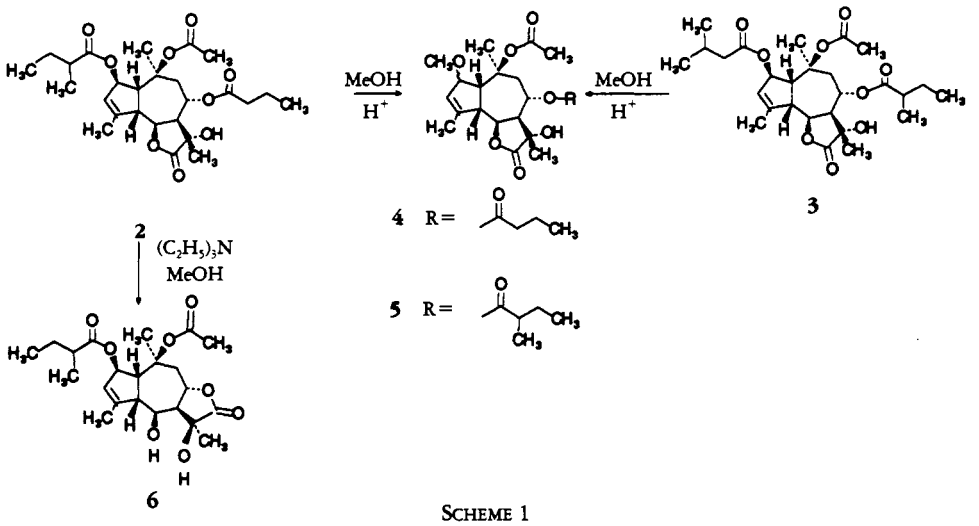
A number of chemotaxonomic studies have focused on the genus *Thapsia* (Apiaceae) because of the presence of thapsigargin [**1**] and other tumor-promoting skin irritants in some of the species (1–9). More detailed studies have revealed a marked heterogeneity of the species *Thapsia villosa* reflected in the morphology, the pattern of secondary metabolites, and in chromosome numbers (3). According to *Flora Europaea* (10), *Thapsia transtagana* Brot. is synonymous with *Thapsia garganica* L., although specimens classified in the two species show different anatomy of the fruits and pattern of secondary metabolites. A characteristic of esterfied guaianolides isolated from *T. garganica* is attachment of a butanoyl group to O-8 (1,2). This location of the butanoyl group has been established for thapsigargin and three analogues differing only by the structure of the acyl groups attached to O-2 and has been verified for the guaianolide **2** (1,2), which is easily converted into an azulene. Our studies on the essential oils of *Thapsia* species established that steam distillation of the fruits of *T. transtagana* gave a blue oil that contained two main components: eugenol methyl ether (47.2%) and 1,4-dimethylazulene (52.8%). The proazulene **3** of *T. transtagana* was isolated from fruits as well as roots. Comparison of the ^{1}H-nmr and ^{13}C-nmr spectra of **2** (2) with those of **3** revealed only small differences, which could be accounted for by assuming that the butanoyl group in **2** had been replaced with a 3-methylbutanoyl group in **3**. Inspection of the cims further confirmed that the molecular ion of **3** was 14 units heavier than the molecular ion of **2**. These considerations, however, gave no evidence for the locations of the acyl groups. Because the acyl groups of **2** have never been located unequivocally (2), we took the opportunity of isolation of a new analogue to answer this question.

Attempts to locate the acyl groups by COLOC spectroscopy (11–13) failed because



1

no three-bond couplings between H-2 or H-8 and any of the carbonyl carbons could be observed. The acyl groups were instead localized through partial hydrolysis and by interpretation of the mass spectra of the methyl ethers **4** and **5**. Heating of an MeOH solution of **2** or **3** after addition of 5% trifluoroacetic acid leads to formation of the methyl ether **4** or **5**, respectively. Since this reaction proceeds by the A_{AL1} mechanism, which is favored when the alcohol is allylic (14), the lost acyl groups (2-methylbutanoyl or 3-methylbutanoyl, respectively) had to be located at O-2 (Scheme 1).



SCHEME 1

In the case of compound **2**, the butanoyl group was located by triethylamine-catalyzed methanolysis. The ^{1}H-nmr spectrum of the product obtained revealed that the butanoyl group was lost, a conclusion that was further confirmed by the molecular weight as determined by cims. However, the ^{1}H-nmr spectrum disclosed that the product was not simply the secondary alcohol, which would have been obtained by solvolysis of the butyric ester. Instead the spectrum suggested the compound to possess the structure **6**, which is formed by a relactonization after the solvolysis. Unexpectedly, however, the signal assigned to H-6 appeared as a singlet in spite of attachment of a hydrogen to both of the neighboring carbons. The absence of strong coupling to H-6 might be explained by assuming that the torsional angle between H-6 and H-5 as well as that between H-6 and H-7 is approximately 90°. Force fields calculation using PC modeling (15) confirmed that such a conformation is a local energy minimum, and the calculations predicted coupling constants of less than 1 Hz. Formation of compound **6** proved the butanoyl group to be attached to O-8. The remaining acetyl group might be located at O-10 or O-11. Comparison of ^{1}H-nmr spectra of guaianolides possessing the oxygenation pattern and stereochemistry of **2** and **3** discloses that the chemical shift value of H-7 is approximately 3.6 if O-8 as well as O-11 is acylated and 3.0 if only O-8 is acylated (2,16). In the case of **2** and **3**, the δ values of H-7 were 3.0, indicating a free hydroxy group at C-11.

In the case of **3**, attempts to localize an acyl group by triethylamine-catalyzed solvolysis failed. This result by itself indicates that the sterically hindered 2-methylbutanoyl is attached to O-8 and that the acetyl group consequently is attached to O-10. Further evidence for this conclusion was obtained by comparison of the eims of **4** and **5**. In both spectra the acetyl group was found to be eliminated as HOAc far more easily than the other acyl group (butanoyl and 2-methylbutanoyl, respectively). This was

revealed by the presence of a peak at *m/z* 332 (5%) $[M-92]^+$ in the spectrum of **4** and a peak at *m/z* 346 (3%) $[M-92]^+$ in the spectrum of **5**. These peaks are assigned to the ion obtained by elimination of HOAc and MeOH from the molecular ions. The elimination of MeOH from methyl ethers is a general process that becomes more prominent by low energy ionization of the molecule (17). In the spectra of **4** and **5**, no peaks corresponding to elimination of either butanoic acid or 2-methylbutanoic acid, respectively, or butanoic acid plus MeOH or 2-methylbutanoic acid plus MeOH, respectively, were observed. Similar fragmentation patterns in the spectra of **4** and **5** indicate that the acetyl group is attached to the same oxygen in both guaianolides. The esterification of HOAc to a tertiary alcohol might explain why this acid is eliminated more readily than the other.

An interesting feature was revealed by comparison of the locations of the acyl groups in the three types of oxygenated guaianolides previously isolated from *T. garganica*. In all cases butanoic acid, an acid that is seldom esterified to guaianolides, is attached to O-8 (18,19). Analogously, 2-methylbutanoic acid is always found to be esterified to O-8 in the varying types of oxygenated guaianolides isolated from *T. transtagana* (20,21). This consistency might indicate divergent biogeneses of the esterified oxygenated guaianolides which, combined with the different compositional pattern of fruit volatiles (22) and genetic and morphological studies, might give a sound basis for dividing the species *T. garganica* into more species, e.g., *T. garganica* and *T. transtagana.*

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Cc was performed over Si gel, type 60 (Merck). Tlc was carried out on Merck aluminium-backed tlc sheets (Si gel F_{254}). The tlc systems employed were CH_2Cl_2-EtOAc (19:1), toluene-EtOAc-MeOH (30:8:1), hexane-EtOAc (3:1), and toluene-EtOAc (4:1). Spots were visualized by spraying with either 1M H_2SO_4 or a 1% solution of 1,3-dihydroxynaphtalene in 1M ethanolic H_2SO_4. Ms analyses in the ci (CH_4) and dci (NH_4) mode were carried out on a Finnigan MAT 8200 mass spectrometer equipped with a super INCAS data system. Gc-ms was performed with a Hewlett-Packard HP 5995 C spectrometer interfaced with an HP 599704 data system. The ion source was operating in the ei mode and the sample injected using the splitting technique. The apparatus was fitted with an HP-1 fused Si capillary column, 12 m×0.2 mm, id 0.33 μm. Nmr spectra were recorded on a Bruker AMX 400 and a Bruker AF 200 instrument. Standard pulse sequences were used for COSY, COLOC, and refocused INEPT. In order to optimize the polarization transfer from ^1H to ^{13}C during the COLOC experiment, the $^3J_{HC}$ value was determined by a series of refocused INEPT spectra (13).

PLANT MATERIAL.—Fruits and roots from *T. transtagana* [2n=22 (2x)] were collected by UWS in the summer 1981 and 1988, respectively. Voucher specimens (81–20, collected at Vilamoura, Portugal; 88–28, collected 2 km west of Sta. Luzia, Portugal) are registered in the Department of Pharmacognosy, Royal Danish School of Pharmacy.

FRUIT VOLATILES FROM *T. TRANSTAGANA*.—The fruits (10 g) were steam-distilled for 3 h and the volatiles extracted according to Avato *et al.* (23). The components were analyzed and identified by gc-ms (23).

ISOLATION OF **3**.—The dried fruits of *T. transtagana* (10 g) were mechanically crushed to a fine powder, which was extracted under stirring with EtOH for 36 h. The extract was concentrated in vacuo and the residue partitioned between H_2O and EtOAc. The organic phase was concentrated in vacuo, and **3** (8 mg) was isolated from the residue by cc using mixtures of CH_2Cl_2/EtOAc of increasing polarities as eluents. Data for **3**: dcims *m/z* (rel. int.) $[M+NH_4]^+$ 526 (100); cims *m/z* [ion] (rel. int.) $[M+1-HOAc]^+$ 449 (5), $[M+1-C_4H_9COOH]^+$ 407 (3), $[M+1-HOAc-C_4H_9COOH]^+$ 347 (15), $[M+1-HOAc-C_4H_9COOH-C_4H_9COOH]^+$ 245 (28), 41 (100); ^1H nmr see Table 1; ^{13}C nmr see Table 2. Compound **3** could be isolated from the roots by the procedure described by Smitt *et al.* (2).

TRIETHYLAMINE-CATALYZED METHANOLYSIS OF **2**.—A solution of **2** (52 mg, 105 μmol) in MeOH triethylamine (5%) was stirred at 60° for 4.5 h. The reaction mixture was concentrated in vacuo to give a gum, from which **6** (4.61 mg, 10%) and unreacted **2** (19.3 mg, 37%) were isolated by cc using CH_2Cl_2-EtOAc (6:1) as an eluent. Data for **6**: dcims *m/z* (rel. int.) $[M+NH_4]^+$ 442 (100); ^1H nmr see Table 1; ^{13}C nmr see Table 2.

TABLE 1. ^{1}H-nmr Spectra of Compounds **3–6** ($CDCl_3$, TMS).[a]

Proton	Compound			
	3	**4**	**5**	**6**
H-1	3.25 dd (7.5, 3.2)	3.06 m	3.02 m	3.80 dt (7.7, 7.9, 1.5)
H-2	5.70 m	4.23 s	4.23 s	5.69 m
H-3	5.53 m	5.69 m	5.66 m	5.43 d (1.7)
H-5	3.08 m	3.06 m	3.02 m	2.97 m
H-6	4.60 dd (11.7, 9.2)	4.60 t (9.4)	4.60 t (10.6)	4.15 s
H-7	3.00 t (9.8)	3.03 t (10)	3.02 t (10)	1.51 d (11.0)
H-8	5.70 m	5.69 broad t (<1, 11)	5.66 dt (<1, 11)	5.13 dt (11.1, 4.3)
H_a-9	2.55 dd (15.4, 2.2)	2.66 dd (15.7, <1)	2.66 dd (15.4, <1)	1.96 dd (14.1, 11.0)
H_b-9	[b]	2.08 dd (15.7, 11.2)	2.09 dd (15.7, 11.3)	2.78 ddd (14.1, 4.7, 1.5)
H-13	1.57 s	1.56 s	1.56 s	1.61 s
H-14	1.36 s	1.41 s	1.42 s	1.39 s
H-15	1.89 broad s	1.90 s	1.90 s	1.81 s

[a]Data are δ (ppm), multiplicity, and *J* (in parentheses) in Hz. The signals originating in the acyl groups are found at: acetyl 2.06; butanoyl 2.29 (t), 1.65 (m), 0.93 (t); 2-methylbutanoyl 2.32 (m), 1.70 (m), 1.45 (m), 1.11 (d), 0.92 (t); 3-methylbutanoyl 2.2 (m), 0.87 (d). The signal originating in the methoxy group in **4** and **5** was found at 3.32 (s).

[b]Overlapped by the signal from the 2-protons in the 3-methylbutanoyl group.

TRIFLUOROACETIC ACID CATALYZED METHANOLYSIS OF **2** AND **3**.—A solution of each of the compounds (10 mg) in a 5% solution of TFA in MeOH was left overnight at 60°. Removal of the solvent in vacuo yielded a greenish oil from which the methyl ethers **4** and **5** were isolated in yields of about 50% by cc using toluene-

TABLE 2. ^{13}C-nmr Data for Compounds **3** and **6** ($CDCl_3$, TMS).[a]

Carbon	Compound	
	3	**6**
C-1	49.2	52.0
C-2	[b]	80.5
C-3	126.6	126.0
C-4	148.9	144.9
C-5	52.3	53.5
C-6	78.8	[b]
C-7	53.4	57.0
C-8	64.8	66.7
C-9	43.4	41.0
C-10	80.8	82.2
C-11	73.2	73.8
C-12	178.5	176.8
C-13	17.2	15.5
C-14	22.2	21.9
C-15	26.5	28.6

[a]Data are δ (ppm). The signals originating in the acyl groups are found at: acetyl 170.4, 22.3; 2-methylbutanoyl 175.7, 41.2, 26.6, 16.4, 11.6; 3-methylbutanoyl 172.5, 43.8, 28.0, 22.1.

[b]Overlapped by the signal from $CDCl_3$.

EtOAc (4:1) as an eluent. Data for **4**: ^{1}H nmr see Table 1; dcims (rel. int.) $[M+NH_4]^+$ 442 (100); gc-ms *m/z* (rel. int.) $[M-MeOH-HOAc]^+$ 332 (5), $[M-MeOH-HOAc-MeCH_2CH_2COOH]^+$ 244 (56), $[M-MeOH-HOAc-MeCH_2CH_2COOH-MeCOCO]^+$ 173 (23), [1,4-dimethylazulene]$^+$ 156 (40), $[Ac]^+$ 43 (100). Data for **5**: ^{1}H nmr see Table 1; Dcims (rel. int.) $[M+NH_4]^+$ 456; gc-ms *m/z* (rel. int.) $[M-HOAc]^+$ 378 (3), $[M-MeOH-HOAc]^+$ 346 (3), $[M-MeOH-HOAc-MeCH_2(Me)CHCOOH]^+$ 244 (29), $[M-MeOH-HOAc-MeCH_2(Me)CHCOOH-MeCOCO)^+$ 173 (50), [1,4-dimethylazulene]$^+$ 156 (23), $[Ac]^+$ (43), (100).

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