Phytochemistry, Vol. 23, No. 4, pp. 809–811, 1984.
Printed in Great Britain.

0031–9422/84 $3.00 + 0.00

(8*R*,14*S*)-8-ANGELOYLOXYTHAPSAN-14-OL, A SESQUITERPENE WITH A NOVEL CARBON SKELETON, FROM *THAPSIA VILLOSA*

ELSE LEMMICH, BIRTHE JENSEN and ULLA RASMUSSEN

Departments of Chemistry BC and Pharmacognosy, Royal Danish School of Pharmacy, 2 Universitetsparken, DK-2100 Copenhagen, Denmark

(*Received* 26 *July* 1983)

Key Word Index—*Thapsia villosa*; Umbelliferae; new sesquiterpene; hemiacetal; novel carbon skeleton; thapsan; (8*R*,14*S*)-8-angeloyloxythapsan-14-ol.

Abstract—The roots of *Thapsia villosa* afforded in addition to other terpenoids, a sesquiterpene, with a new carbon skeleton. The structure, including the relative configuration, was elucidated by spectroscopic methods, a few chemical transformations and by X-ray analysis. The absolute configuration was determined by means of the method of Horeau. The trivial name thapsan is suggested for the parent structure [2a*R*,2b*R*,6a*S*,7a*S*]-2b,3,3,6a-tetramethylperhydroindeno[2,3-c]-furan.

INTRODUCTION

Recent chemotaxonomic investigations have revealed the presence within the genus *Thapsia*, of a number of sesquiterpenes, mostly sesquiterpene lactones. These lactones are tri- or tetraesters of 3,7,8,10,11-pentahydroxy-C_6-guaianolide or 2,3,7,8,10,11-hexahydroxy-C_6-guaianolide [1], which have been shown to be highly potent histamine liberators [2], and thus responsible for the well known skin irritating activity of some *Thapsia* species. The present study concerns plant material of *T. villosa* L., which was shown to be rather heterogeneous. Based on morphological and anatomical characters, as well as chemical constituents, the material could be divided into at least four groups [3]. The plants from one of these groups have morphological characters equal to the former species *T. minor* Hoff. & Link and show no skin irritating activity. From an ethanol extract of the roots was isolated guaiol and a new sesquiterpene (**1**). The structure elucidation of the latter compound is reported in this communication.

RESULTS AND DISCUSSION

Compound **1** was obtained as a crystalline material, which analysed for $C_{20}H_{32}O_4$. The IR spectrum showed the presence of a hydroxyl group (3400 cm^{-1}), an ester group (1700 cm^{-1}) and a double bond (1650 cm^{-1}). Characteristic chemical shift data and coupling patterns in the ^1H NMR spectrum (Table 1) and in the ^{13}C NMR spectrum (Table 2) together with the EI-mass spectrum ($[M-100]^+$) showed that **1** was an angelate. As no further double bonds were observed from the ^{13}C NMR spectrum it could be concluded that **1** was tricyclic. The hemiacetal grouping of **1** was indicated by a carbon signal at 100 ppm in the ^{13}C NMR spectrum and, in the ^1H NMR spectrum, by a hydroxyl group and a one proton doublet at δ5.4, which was changed into a singlet by deuterium exchange. By oxidation of **1** with silver carbonate a lactone (**2**) was formed. Compound **2** was evidently a γ-lactone (IR 1765 cm^{-1}) and it could thus be concluded that the hemiacetal grouping in **1** was part of a five membered ring. Reduction of **1** with sodium borohydride resulted in a product with two primary hydroxylic groups instead of the hemiacetal grouping (**3**, Tables 1 and 2). The presence of an isolated 5-proton spin system in **1** corresponding to the partial structure **4** could be deduced from the ^1H NMR spectrum. Decoupling experiments revealed that one of these protons, situated in a methine group (δ2.8) couples with each of the four others: a

Table 1. ^1H NMR spectral data of compounds **1**, **2**, **3** and **7** (270 MHz, $CDCl_3$, TMS as internal standard)

	1	2	3	7
H-1				
H-2	1.2–1.5 *br*	1.2–1.5 *br*	1.2–1.5 *br*	1.2–1.6 *br*
H-3				
H-6	3.02 *d*	3.37 *d*	2.60 *m*	2.86 *d*
H-7	2.80 *dddd*	2.97 *ddd*		2.65 *dddd*
H-8	4.80 *d*	4.75 *d*	4.98 *d*	3.64 *d*
Me	0.91 *s*	1.00 *s*	0.81 *s*	0.84 *s*
Me	0.92 *s*	1.03 *s*	0.95 *s*	0.89 *s*
Me	0.98 *s*	1.12 *s*	0.96 *s*	0.96 *s*
Me	1.04 *s*	1.14 *s*	0.99 *s*	1.05 *s*
H-14	5.39 *s**			5.35 *s**
H-15α	4.19 *dd*	4.52 *d*	3.80 *m*	4.19 *dd*
H-15β	4.25 *dd*			3.90 *dd*
	6.05 *qq*	6.12 *qq*	6.08 *qq*	
OAng	1.92 *dq*	1.99 *dq*	1.93 *dq*	
	1.89 *dq*	1.89 *dq*	1.90 *dq*	

s* upon addition of D_2O. *J* (Hz) compound **1: 6,7 = 11; 6,14 not measurable; 7,8 = 7; 7,15α = 8; 7,15β = 3; 15α,15β = 9; compound **2**: 6,7 = 13; 7,8 = 9; 7,15 = 7; compound **3**: 7,8 = 7; compound **7**: 6,7 = 11; 6,14 not measurable; 7,8 = 8; 15α = 8; 15α,15β = 7.

Table 2. ^{13}C NMR spectral data of compounds **1**, **2**, **3** and **7** (22.5 MHz for **1** and 67.9 MHz for **2**, **3** and **7**; $CDCl_3$)

	1	2	3	7
C-1	30.6 *t*	31.2 *t*	31.2 *t*	29.3 *t*
C-2	17.9 *t*	18.0 *t*	17.8 *t*	17.9 *t*
C-3	37.5 *t*	38.5 *t*	38.3 *t*	37.8 *t*
C-4	35.8 *s*	36.1 *s*	36.5 *s*	36.1 *s*
C-5	47.0 *s**	50.8 *s**	45.5 *s**	48.4 *s**
C-6	55.4 *d*	48.4 *d*†	45.0 *d*†	54.4 *d*
C-7	45.4 *d*	43.6 *d*†	43.9 *d*†	47.3 *d*
C-8	88.1 *d*	88.8 *d*	81.2 *d*	86.8 *d*
C-9	48.8 *s**	49.6 *s**	48.4 *s**	48.4 *s**
C-10	20.0 *q*†	20.4 *q*‡	20.4 *q*‡	20.2 *q*†
C-11	24.3 *q*†	24.5 *q*‡	25.2 *q*‡	24.4 *q*†
C-12	27.6 *q*	30.5 *q*	29.3 *q*	27.4 *q*
C-13	13.2 *q*	15.2 *q*	14.3 *q*	13.4 *q*
C-14	100.3 *d*	177.2 *s*	61.7 *t*	100.5 *d*
C-15	71.2 *t*	72.3 *t*		70.8 *t*
OAng	168.1 *s*	168.4 *s*	168.6 *s*	
	127.9 *s*	127.5 *s*	127.4 *s*	
	137.7 *d*	139.4 *d*	138.4 *d*	
	15.7 *q*	16.0 *q*	15.9 *q*	
	20.6 *q*	20.7 *q*	20.7 *q*	

*, †, ‡ Assignments may be interchangeable.

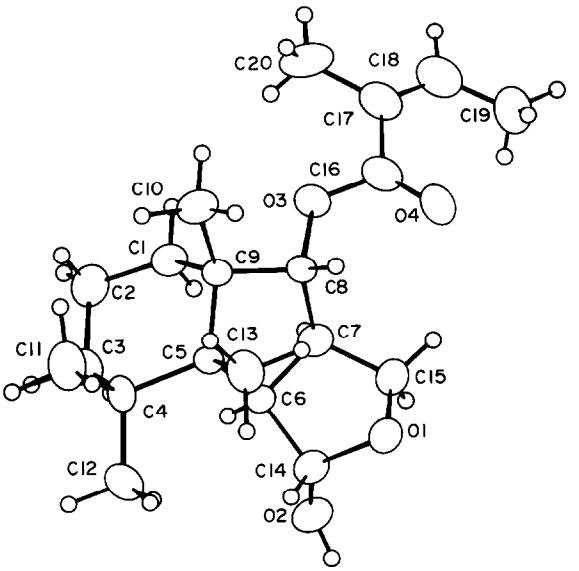
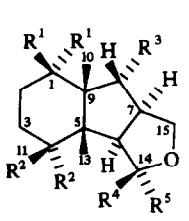
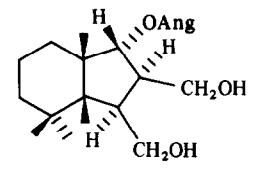


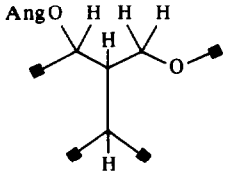
Fig. 1. A view of the molecular structure of compound **1**, including the atomic numbering. Non hydrogen atoms are represented by their thermal ellipsoids, scaled to include 50% probability. The drawing was produced by ORTEP [11].



	R^1	R^2	R^3	R^4	R^5
1	H	Me	OAng	H	OH
2	H	Me	OAng	= O	
5	Me	H	OAng	H	OH
6	H	Me	H	H	H
7	H	Me	OH	H	OH



3



4

methine proton at δ3.02, two diastereotopic protons of a methylene group (δ4.19 and 4.25, J_{gem} = 7 Hz) and a methine proton (δ4.8) situated α to the angeloyloxy group. Taking into account the four methyl singlets in the ^1H NMR spectrum and the signals from three quaternary and three high field methylene carbons in the ^{13}C NMR spectrum, the structural possibilities were **1** and **5** and some stereoisomers of these structures.

The relative configuration at C-6, C-7, C-8 and C-14 could be established from the coupling constants in the ^1H NMR spectrum. To elucidate the configuration at C-5 and C-9 and to locate the gem-dimethyl groups an X-ray analysis was undertaken and this finally proved the structure to be **1** (Fig. 1).

A determination of the absolute configuration of C-14 in **1** was carried out by the empirical method described by Horeau [4], who has shown the method to be applicable to ordinary secondary alcohol groups as well as to hemiacetal ones [5]. The configuration at C-14 was found to be *S* and choosing [2a*R*,2b*R*,6a*S*,7a*S*]-2b,3,3,6a-tetra-

Table 3. Final positional parameters of **1**

Atom	*x*	*y*	*z*
C-1	0.3568 (5)	0.5415 (7)	0.3854 (4)
C-2	0.4988 (5)	0.4776 (8)	0.4065 (4)
C-3	0.5008 (5)	0.3194 (7)	0.3352 (4)
C-4	0.4490 (4)	0.3458 (−)	0.1989 (4)
C-5	0.3072 (4)	0.4312 (6)	0.1703 (3)
C-6	0.2007 (4)	0.3082 (6)	0.1968 (3)
C-7	0.0965 (5)	0.4124 (6)	0.2402 (4)
C-8	0.1419 (5)	0.5926 (6)	0.2320 (4)
C-9	0.2934 (4)	0.5840 (6)	0.2522 (4)
C-10	0.3454 (5)	0.7536 (7)	0.2226 (5)
C-11	0.5527 (5)	0.4502 (8)	0.1543 (5)
C-12	0.4423 (5)	0.1755 (7)	0.1390 (4)
C-13	0.2703 (5)	0.4882 (7)	0.0373 (4)
C-14	0.1165 (4)	0.2053 (6)	0.0920 (4)
C-15	−0.0363 (5)	0.3641 (7)	0.1571 (4)
C-16	−0.0069 (6)	0.7779 (7)	0.2952 (4)
C-17	−0.0149 (6)	0.9071 (8)	0.3889 (5)
C-18	−0.1271 (7)	0.9613 (9)	0.3979 (5)
C-19	−0.2590 (7)	0.9121 (12)	0.3296 (6)
C-20	0.1176 (8)	0.9717 (10)	0.4673 (6)
O-1	−0.0031 (3)	0.3003 (5)	0.0511 (2)
O-2	0.0914 (3)	0.0499 (4)	0.1974 (3)
O-3	0.1116 (3)	0.7034 (5)	0.3218 (3)
O-4	−0.0932 (4)	0.7410 (6)	0.2086 (3)
H (O-2)	0.058	−0.021	0.077

methylperhydroindeno[2,3-c]furan (**6**) as an appropriate parent structure, for which we suggest the name thapsan, the semi-systematic name [8*R*,14*S*]-8-angeloyloxythapsan-14-ol may be given to **1**. The parent alcohol **7** obtained by saponification of **1** was also characterized (see Experimental section).

EXPERIMENTAL

Plant material. Root material of *T. villosa* L. was collected in Portugal on 26 June 1977, 5 km east of Capo Espichel road no. 379. Voucher specimens are being kept in the Department of Pharmacognosy, Royal Danish School of Pharmacy, Copenhagen.

Extraction and purification. Extraction and the first fractionation has been described earlier [2]. Further purification was carried out on silica gel with toluene–EtOAc (2:1), to which MeOH (0–2%) was added, as eluent, and finally on silica gel with petrol, to which Me_2CO (3–30%) was added, as eluent. TLC examination on silica gel, petrol–Me_2CO (4:1), visualization with a mixture of 10% phosphomolybdic acid in EtOH and naphthoresorcinol-R (1:1).

Guaiol. Mp 89.5–90.5° (EtOH–H_2O) (lit. [6, 7] 91°). $[\alpha]_D^{25}$ −25.8° (EtOH 96%, *c* 1) [lit. [6, 7]: $[\alpha]_D^{25}$ −26.64° (EtOH 95%)]. IR data in accordance with those published for guaiol [6, 7].

[8R,14S]-8-*Angeloyloxythapsan*-14-*ol* (**1**). Obtained as a crystalline compound, mp 159–161° (petrol–Me_2CO). (Found: C, 71.49; H, 9.45. $C_{20}H_{32}O_4$ requires: C, 71.39; H, 9.59%). $[\alpha]_D^{23}$ −8.8°, $[\alpha]_{436}^{23}$ −12.7° ($CHCl_3$, *c* 1). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1700 (>C=C–CO·OR), 1650 (>C=C<). EIMS (probe) 70 eV, *m/z* (rel. int.): 336 $[M]^+$ (7), 319 $[M-2-Me]^+$ (2), 290 (58), m* 336 → 290 and 334 → 290, 237 $[M-AngO]^+$ (9), 236 $[M-AngOH]^+$ (40), 218 (3), m* 236 → 218, 191 (33), 190 (98), m* 236 → 190, 83 (97), 55 (100).

[8R]-8-*Angeloyloxythapsan*-14-*one* (**2**). To a soln of 25 mg **1** in C_6H_6 (4.5 ml) was added 500 mg silver carbonate on Celite (Fetizon's reagent [8]) and the mixture was refluxed for 1.5 hr. TLC examination showed only one product. After purification on silica gel (20 g) 20 mg crystalline **2** was obtained, mp 146–147° (petrol–Me_2CO). $[\alpha]_D^{23}$ +26.2°, $[\alpha]_{436}^{23}$ +51.2° ($CHCl_3$, *c* 0.5). IR ν_{max}^{KBr} cm^{-1}: 1765 (5-ring lactone), 1700 (>C=C–CO·OR), 1650 (>C=C<). EIMS (probe) 70 eV, *m/z* (rel. int.): 334 $[M]^+$ (30), 319 $[M-Me]^+$ (4), 316 $[M-H_2O]^+$ (2), m* 334 → 316, 278 (1), m* 334 → 278, 251 (3), m* 334 → 251, 235 $[M-AngO]^+$ (11), 234 $[M-AngOH]^+$ (17), m* 334 → 234, 83 (100).

[1R,2R,3R,3aR,7aS]-1-*Angeloyloxy*-2,3-*bishydroxymethyl*-3a,4,4,7a-*tetramethylperhydroinden* (**3**). To a soln of 20 mg **1** in EtOH (4 ml) was added 100 mg $NaBH_4$ and the mixture was left at room temp. for 48 hr. TLC examination showed only one product. After neutralization with HCl and dilution with H_2O the product was extracted with Et_2O and purified on silica gel (20 g). Crystalline **3** (15 mg) was obtained, mp 124–125° (petrol–Me_2CO). $[\alpha]_D^{23}$ +29.8°, $[\alpha]_{436}^{23}$ +61.2° ($CHCl_3$, *c* 0.5). IR ν_{max}^{KBr} cm^{-1}: 3400, 3300 (OH), 1710 (>C=C–CO·OR), 1650 (>C=C<). EIMS (probe) 70 eV, *m/z* (rel. int.): 338 $[M]^+$ (0.4), 320 $[M-H_2O]^+$ (0.5), 308 (6), 307 $[M-31]^+$ (3), 290 (0.7), m* 308 → 290, 289 (3), m* 307 → 289, 239 $[M-AngO]^+$ (3), 238 $[M-AngOH]^+$ (4), 220 $[320-AngOH]^+$ (9), m* 320 → 220, 208 $[308-AngOH]^+$ (20), 83 (100).

[8R,14S]-*Thapsan*-8,14-*diol* (**7**). To 14 mg **1** was added 1 M NaOMe (1.5 ml) and the mixture was kept at 60° for 2 hr. After neutralization with solid CO_2 the reaction mixture was extracted with Et_2O and purified on silica gel (10 g). Crystalline **7** (9 mg) was obtained, mp 158–160° (petrol–Me_2CO). $[\alpha]_D^{25}$ −58.4°, $[\alpha]_{436}^{25}$ −113.0° ($CHCl_3$, *c* 0.5). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH). EIMS (probe) 70 eV, *m/z* (rel. int.): 254 $[M]^+$ (0.3), 253 $[M-1]^+$ (0.2), 237 $[M-2-Me]^+$ (2), 236 $[M-H_2O]^+$ (5), 208 (40), 193 $[208-Me]^+$ (6), m* 208 → 193, 190 $[208-H_2O]^+$ (29), m* 208 → 190, 175 $[190-Me]^+$ (14), m* 190 → 175, 151 (56), m* 208 → 151, 82 (100).

Crystal data. [8*R*,14*S*]-8-Angeloyloxythapsan-14-ol, $C_{20}H_{32}O_4$, M = 336.5. Monoclinic, space group $P2_1$, a = 10.443 (7), b = 8.015 (4), c = 11.596 (4) Å, β = 104.02 (4)°; V = 941.6 Å^3, Z = 2, D_c = 1.19 g cm^{-3}, μ (Mo$K\alpha$) = 0.80 cm^{-1}. F (000) = 368. Three dimensional diffraction data were measured at room temp. on a Nonius CAD-3 diffractometer using standard techniques. The structure was solved and refined using MULTAN-77 [9] and the programs of the X-Ray-system [10], respectively. Detailed information about experimental conditions and the refinements are, together with the final lists of structure factors, thermal parameters and calculated positional parameters for hydrogen atoms, available from the authors on request.

Determination of the absolute configuration at C-14 by the Horeau method. Compound **1** (35.4 mg) was esterified with racemic α-phenylbutyric anhydride using the standard method of Horeau [5]. The optical rotation of the liberated α-phenylbutyric acid was corresponding to an optical purity of 53%. The calculated epimeric ratio was 3.25:1. A ^1H NMR spectrum of the mixture of the epimeric α-phenylbutyric esters of **1**, which had been formed during the reaction, confirmed this ratio.

Acknowledgements—The authors are grateful to members of Chemical Laboratories II and V, University of Copenhagen for recording MS and NMR spectra. Thanks are due to the Danish Natural Science Research Council for the provision of NMR and X-ray facilities.

REFERENCES

1. Christensen, S. B. and Schaumburg, K. (1983) *J. Org. Chem.* **48**, 396.
2. Rasmussen, U., Christensen, S. B. and Sandberg, F. (1978) *Acta Pharm. Suec.* **15**, 133.
3. Rasmussen, U., Christensen, S. B. and Sandberg, F. (1981) *Planta Med.* **43**, 336.
4. Horeau, A. (1961) *Tetrahedron Letters* 506.
5. Horeau, A. and Kagen, H. B. (1964) *Tetrahedron* **21**, 2431.
6. Gandurin, A. (1908) *Ber.* **41**, 4359.
7. Morris, W. W. (1973) *J. Ass. Off. Analyt. Chem.* **56**, 1037.
8. Fetizon, M. and Golfier, M. (1968) *C. R. Acad. Sci., Paris, Ser. C* **267**, 900.
9. Main, P., Lessinger, L., Woolfson, M. M., Germain, G., and Declercq, J.-P. (1977) MULTAN, *A System of Computer Programs for the Automatic Solution of Crystal Structures from X-Ray Diffraction Data.* Universities of York, England and Louvain, Belgium.
10. Stewart, J. M., Kruger, G. J., Ammon, H. L. Dickinson, C., and Hall, S. R. (1972) The *X-Ray System*, Version of June 1972, Technical Report TR-192, Computer Science Center, University of Maryland, College Park 1972.
11. Johnson, C. K. (1971) *ORTEP II, A Fortran Thermal Ellipsoid Plot Program for Crystal Structure Illustrations, revised.* Oak Ridge National Laboratory, Oak Ridge.