Tetrahedron Letters,Vol.26,No.1,pp 107-110,1985
Printed in Great Britain

0040-4039/85 $3.00 + .00

ABSOLUTE CONFIGURATIONS OF THE HISTAMINE LIBERATING SESQUITERPENE LACTONES THAPSIGARGIN AND TRILOBOLIDE

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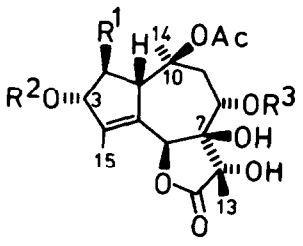
Summary: *The absolute configuration of trilobolide and the revised one of thapsigargin classify the compounds as a group of guaianolides previously not found in higher plants.*

A number of very potent skin irritating sesquiterpene lactones, including thapsigargin (1) and thapsigargicin (2), have been isolated from the roots of *Thapsia garganica* (L)[1]. Based on a X-ray analysis of the epoxide (3)[2] and chemical and spectroscopical investigations[3], the relative configurations shown by formulae 1 and 2 were assigned to thapsigargin and thapsigargicin, respectively.

Expansion of the phytochemical investigations of *Thapsia garganica L. (Apiaceae)* to the entire genus *Thapsia* revealed the presence of a number of lactones, all having the same hexaoxygenated guaianolide nucleus as in 1 and 2[4,5]. In addition, two pentaoxygenated guaianolides were isolated[4], one of which was identical to trilobolide (4), previously isolated from *Laser trilobum* (L.) BORKH[6] *(Apiaceae)*. Beside constituting an interesting phytochemical group, these sesquiterpene lactones also possess a number of very interesting pharmacological properties. Thus, they are very potent histamine liberators from rat mast cells[1] and activate human neutrophilic and basophilic leucocytes[7]. A screening of 2 against P388 lymphocytic leukemia performed under the auspices of the Development Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland, has disclosed a T/C value of 135 at 4 mg/kg.

Application of Horeau's method to the secondary alcohol 5, obtained by partial saponification of 1, indicated that 5 and consequently 1 possess absolute configurations opposite to those shown in formulae 5 and 1 respectively[3]. A major drawback of Horeau's method, however, is that priority of the ligands bound to the asymmetric alcohol carbon can only be assigned on an empirical basis[8]. Wrong choices of priority have led in some cases, also in the field of sesquiterpene lactones, to wrong conclusions concerning the absolute configurations[9]. The presence in 1 of an allylic alcohol moiety esterified with an α,β-



1: R^1=Oc; R^2=An
R^3=Bu

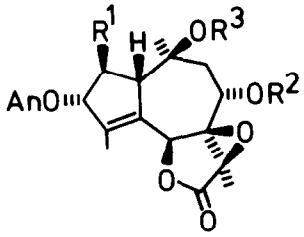
2: R^1=Hx; R^2=An
R^3=Bu

4: R^1=-H; R^2=An
R^3=*S*-MBu

5: R^1=Oc; R^2=An
R^3=-H

6: R^1= Oc; R^2=MBu
R^3=Bu

7: R^1=-H; R^2=MBu
R^3=*S*-MBU



3: R^1=Oc; R^2=Bu
R^3=Ac

8: R^1=-H; R^2=*S*-MBu
R^3=Ac

9: R^1=R^2=R^3=-H

10: R^1=R^2=-H
R^3=Ac

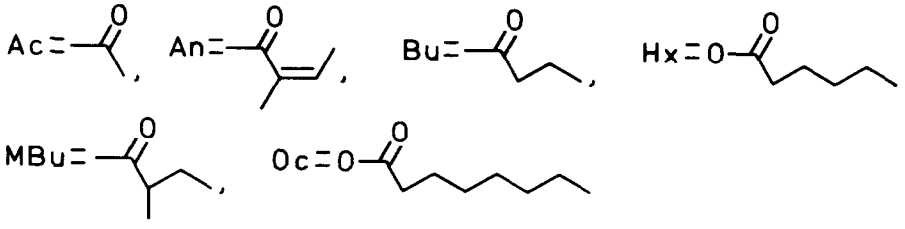
11: R^1=R^3=-H
R^2=*S*-MBu

12: R^1=R^3=-H
R^2=*R*-MBu

Table 1 ^{1}H NMR Data (270 MHz;$CDCl_3$) for the new compounds 6, 7, 11, and 12.

proton	6	7	11	12
H(1)	4.25 br	4.34 br	3.27 br	3.27 br
H(2)	5.6 m	*a*	2.66 m	2.66 m
H(2')	-	*a*	*a*	*a*
H(3)	5.44 m	5.52 m	5.59 m	5.59 m
H(6)	5.6 m	5.68 br	5.24 br	5.24 br
H(8)	5.6 m	5.62 t	4.98 t	4.98 t
H(9)	2.99 dd	3.08 dd	2.27 dd	2.26 dd
H(9')	*a*	2.17 dd	2.20 dd	2.20 dd
H(13)	1.48 s	1.48 s	1.63 s	1.63 s
H(14)	1.41 s	1.33 s	1.20 s	1.20 s
H(15)	1.83 br	1.88 br	1.84 br	1.84 br
MBu				
CH	2.42 m	*a*	2.43 m	2.45 m
2Me	1.18 d	1.18 d 1.15 d	1.19 d	1.18 d
3Me	0.94 t	0.94 t 0.91 t	0.92 t	0.93 t

a: The signal coincided with other signals.



unsaturated acid made it possible to use the non-empiral CD exciton method for determination of the absolute configuration at C(3)[10]. All contributions to the Cotton effects of 1 except those originating from the allylic angeloate moiety could be excluded by comparison of the CD spectra of 1 and 6. The dihydro derivative 6 was prepared by palladium-catalysed hydrogenation of an ethanolic solution of 1 at atmospheric pressure. Only the trisubstituted double bond was affected, and according to 270 MHz ^{1}H NMR spectroscopy, only one of the epimeric dihydroderivatives was formed. Subtraction of the two CD spectra showed that the allylic angeloate system of 1 led to a positive Cotton effect at 217 nm ($\Delta\varepsilon$=+5.4) proving the (S) configuration at C(3), as shown in formula 1. This stereochemistry, however, implies an α-orientated C(7)-C(11) bond, which is unique in guaianolides isolated from higher plants[11]. (It is assumed that the structures are drawn, with the cyclopentene moiety to the left). In order to get further evidence for this stereo-chemistry, based on the CD exciton chirality method, which contradicts the result of Horeau's method, we decided to include trilobolide (4) to our investigations. From a biogenetic point of view it is very likely that 1 and 4 will have the same absolute configuration at C(3), C(6), C(7), C(8), C(10), and C(11). Based on ^{13}C NMR spectroscopic investigations we have previously suggested the relative configuration shown in formula 4 for trilobolide[12], a suggestion which has recently been confirmed by ^{1}H NMR[13] and X-ray analysis[14]. Being aware of the outcome of the Horeau analysis on 1 we choose, however, to draw the enantiomer of formula 4, whereas the other two papers[13,14] show formula 4. Comparison of the Cotton effect at 223 nm for 4 and the dihydroderivative 7 revealed a positive contribution from the allylic angeloate moiety ($\Delta\varepsilon$= + 2.8), as would be expected from the antipode represented by formula 4 (compound 7 was prepared by palladium-catalysed hydrogenation of 4). A further proof for this absolute configuration was obtained by proving the α-carbon in the 2-methylbutanoate moiety to have S-configuration. Based on this knowledge and the relative configuration of the entire molecule as evidenced by the X-ray structure[14], trilobolide could be concluded to have the absolute configuration represented by formula 4. The strategy for determing the absolute configuration was to compare the 270 MHz ^{1}H NMR spectrum of 4 with that of a derivative in which the 2-methylbutanoate group had been replaced with a *S*-2-methylbutanoate group. Attempts to remove the 2-methylbutanoate group by partial saponification of 4 failed, probably because of the β-hydroxylactone moiety. However, treatment of 8[6] with a 5% potassium hydroxide solution for 4 h afforded a mixture of 9(20%) 10(5%), and 11(25%). 4-Dimethylaminopyridine-catalyzed reacylation of 9 with *(S,S)*-2-methylbutyric anhydride afforded a product, the 270 MHz ^{1}H NMR spectrum of which was superimposable with that of 11. In contrast, the spectrum obtained when 9 was reacylated with racemic 2-methylbutyric anhydride clearly contained signals from the two epimeric products 11 and 12. Especially the signals originating from the methine proton and the 2-methyl protons of the 2-methylbutanoate moiety were well separated (Table 1).

Based on the concurrent results of the CD exciton chirality method and the X-ray analysis[14] combined with the determination of the absolute configuration of the 2-methylbutanoate moiety, we conclude that trilobolide does have the absolute configuration shown in formula 4. Furthermore, we revise the absolute configuration of thapsigargin to that of formula 1. From a biogenetic point of view it is very likely that the guaianolide nucleus in all the lactones described in ref. 5 also should be assigned this absolute configuration. These lactones thus are representatives of a group of guaianolides isolated from higher plants possessing an α-orientated C(7)-C(11) bond. In the formula of trilobolide given in a review of sesquiterpene lactones the only asymmetric center, for which an absolute configuration is suggested, is that of C(7)[11]. Based on biogenetic considerations the wrong configuration is suggested illustrating how unexpected the correct stereochemistry is.

Acknowledgement. We are grateful to Dr. G. Borch and Mrs. L. Penzien for recording the CD spectra with a Dichrographe II, Roussel-Jouan, and to Mrs. J.Cohr for recording the NMR spectra with a Bruker HX 270 S. Both instruments are owned by the Danish Natural Science Research Council.

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(Received in UK 4 October 1984)