Phytochemistry, Vol. 24, No. 4, pp. 767–770, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00

THE ACTINIDOLS: NOR-ISOPRENOID COMPOUNDS IN GRAPES, WINES AND SPIRITS

EUGENE DIMITRIADIS, CHRISTOPHER R. STRAUSS, BEVAN WILSON and PATRICK J. WILLIAMS*

The Australian Wine Research Institute, Private Mail Bag, Glen Osmond, South Australia, 5064, Australia

(*Received* 14 *August* 1984)

Key Word Index—*Vitis vinifera*; Vitaceae; grapes; wines; distilled spirits; aroma volatiles; C_{13} nor-isoprenoid; actinidols.

Abstract—The isomeric actinidols, 2,2,6-trimethyl-8-(1-hydroxy)ethyl-7-oxabicyclo[4.3.0]nona-4,9-dienes, have been identified in steam distillates of juices from *Vitis vinifera* grape vars. Muscat of Alexandria, Chardonnay and Doradillo. The two major isomers of this compound, which were also commonly observed in wines and brandies, were tentatively identified as having a *trans*-stereochemistry about the dihydrofuran ring in the molecule.

INTRODUCTION

The presence in grapes of precursors of C_{13} nor-isoprenoid compounds has been established [1, 2]. These precursors are non-volatile components, which can be isolated from juice along with monoterpene glycosides, and which under mild hydrolytic conditions at pH 3 give volatiles, including damascenone and vitispirane [3]. Additionally, an isomeric pair of compounds (previously referred to as peaks 39 [2]) showing a characteristic EIMS dominated by the base peak, *m/z* 163, were also observed among these hydrolysis products.

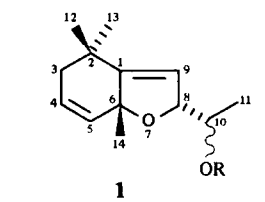
These compounds with base peak *m/z* 163, are of importance because of their occurrence, commonly observed in these laboratories, as volatiles of bottle-aged white wines, brandies and heated muscat grape juices. Recently GC/MS analyses of extracts of steam distillates of juices of *Vitis vinifera* L. grape vars. Muscat of Alexandria, Chardonnay and Doradillo [4] showed that, in addition to the two major isomers usually observed, a further two minor isomers also with base peak *m/z* 163 were present.

This report identifies these four grape-derived compounds as the actinidols (**1a** and **2a**) and presents data assigning the two major naturally occurring compounds as the *trans* isomers (**1a**).

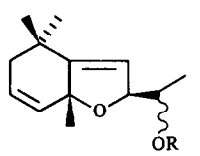
RESULTS AND DISCUSSION

Actinidol was originally isolated from the leaves of *Actinidia polygama* by Sakan *et al.* [5]. Subsequently these workers [6] synthesized actinidol(s) by a route involving photo-oxygenation of dehydroionone, followed by reduction of the photo product to triols **3a** and treatment of these with sulphuric acid. However the stereochemistry of either the natural or synthetic products was not defined and some doubt about the position of the cyclohexene double bond remained [7].

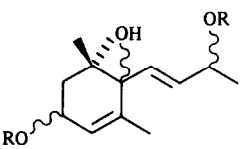
The synthetic approach to the actinidols **1a** and **2a** taken here also utilized diastereoisomeric triols **3a** [6] or rearrangement products (**4**) [Strauss, C. R., Dimitriadis, E., Wilson, B. and Williams, P. J., in preparation]. Heating either diastereoisomeric **3a** or **4** in aqueous acid at pH 3 led to the formation of a similar mixture of volatiles. GC/MS of the fragrant hydrolysis products showed that the four isomeric actinidols were present in a ratio of



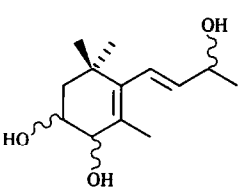
1



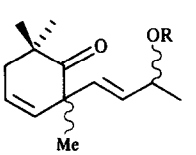
2



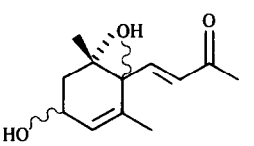
3



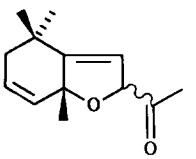
4



5



6



7

a R = H
b R = Ac

No absolute stereochemistry is implied in structures **1**, **2** and **7**.

*To whom inquiries should be directed.

40:53:3:4 and constituted 30–40% of the total volatiles. The major products of the hydrolyses (48%) were ketones **5a** which have also been observed as grape products [Strauss, C. R., Dimitriadis, E., Wilson, B. and Williams, P. J., in preparation].

In order to isolate the hydrolysis products the reaction mixture was acetylated and the actinidyl acetates (**1b** and **2b**) separated from keto acetates (**5b**) by LC. Further chromatography allowed one major actinidyl acetate isomer to be isolated individually whilst the other isomer was obtained in a second fraction, together with a small amount of the minor isomeric pair of acetates. On saponification these two acetate fractions gave the two major, short R_t, actinidols in purities of 95% and 91%.

In an attempt to determine the stereochemistry of each actinidol isomer several experiments were undertaken aimed at securing larger amounts of the minor, long R_t, isomeric pair in order to isolate these compounds and study their individual NMR spectra. The experiments involved the following conditions and substrates: (I) Separate acid hydrolyses of individual diastereoisomers of triols **3a**. Two isomers were obtained by chromatographing ketones **6** into a *cis* and *trans* pair with respect to the hydroxyl substituents, followed by reduction of the ketones to the triols. (II) Acid hydrolysis of diastereoisomeric diacetates **3b**. (III) Lewis acid catalysed rearrangement of diastereoisomeric triols **3a**.

None of these experiments improved the proportion of the minor actinidol pair and reaction (III) led to greater yields of ketones **5a** at the expense of the actinidols. No attempt was made to form ketones 7 by oxidation, even though this procedure, in principle, might have led to a different isomeric proportion of actinidols after epimerisation and reduction. The facile degradation of such dihydrofurfurylmethyl carbinols to give a lactone (actinidiolide in this case) is well known [5, 8, 9]. Because of this, all manipulations of actinidols were carried out with minimum air contact.

Owing to the inaccessibility of two of the four isomeric actinidols only the major, short R_t, isomers were studied.

Evidence for the stereochemical relationship between the two available actinidol isomers was obtained by interconverting the two through inversion of configuration of the hydroxyl function. Separate reaction of the two actinidols under Mitsunobu inversion conditions [10] cleanly converted each short R_t isomer into the other. Accordingly, the major actinidols produced by hydrolysis of triols **3a** and **4** differed only by the configuration of the secondary alcohol function and, therefore, they had a common stereochemistry about the dihydrofuran ring.

The ^{1}H NMR spectral data for the two major actinidols and their acetates, together with shifts induced by addition of Eu(fod)$_3$ to the free alcohols as well as ^{13}C NMR data, are recorded in Table 1.

The spectral data for the two uncomplexed alcohols and their acetates were similar, and although these spectra gave no insight into the stereochemistry of the compounds, they corroborated the gross structure and confirmed that the two were stereoisomers. This supported the results of the Mitsunobu inversion experiment referred to above, and established that the double bonds were in the same positions in both compounds.

Some NMR data have been published [11–15] for stereoisomeric 7-oxabicyclo[4.3.0]non-9-enes with substituents similar to the actinidols. However because of the small differences in chemical shifts observed between *cis* and *trans* isomers about the dihydrofuran ring in the various compounds studied, it was not possible to use these data for assignment of stereochemistry to the available pair of actinidols.

The addition of Eu(fod)$_3$ to the first eluting actinidol induced only a small shift in the cyclohexene ring methylene protons at position 3, whereas the vinyl proton at position 5 was strongly deshielded. Given the expected co-ordination of the metal atom by the two oxygen atoms of the actinidol, this induced vinyl proton shift indicated that the double bond was in the 4,5 position rather than the alternative 3,4 position.

Absence of a Eu(fod)$_3$ shift in the vinyl protons of the second eluting actinidol indicated co-ordination of the metal atom by the hydroxyl function only in this isomer. This was presumably caused by the configuration of the side chain preventing complete co-ordination by both oxygens in that isomer.

The simultaneous deshielding of the methyl group sited at position 6, the vinyl hydrogen at position 5 and the hydrogen at position 8 by the Eu(fod)$_3$ in the first eluting, bis co-ordinating, isomer gave an indication of the stereochemistry of the dihydrofuran ring system in the actinidol pair. Only in the *trans* isomer, with the europium co-ordinated to both oxygens, should the hydrogens in these three positions experience the environmental influence of the metal complex. With the methyl group sited at position 6 and the side chain sited at position 8 *cis* to each other, the bis co-ordinated metal complex should have been removed from both the position 5 vinyl and position 8 hydrogens, being above the plane of the dihydrofuran ring, and thus would not have been expected to deshield these two hydrogens to any significant extent.

Further support for the *trans* assignment was obtained by observation of a nuclear Overhauser effect (NOE) between the methyl group at position 6 and the hydrogen at position 8 in the acetate esters of the available actinidols. This indicated a close spatial relationship between these two groups. In analogous isomeric 7-oxabicyclo[4.3.0]non-9-ene systems when an NOE has been observed, it has been given only by the *trans* isomer [12]. Although the actinidols themselves did not show any significant NOE, the two available acetates in benzene solution showed 10% and 14% enhancement of the signal for the proton at position 8 when the methyl at position 6 was irradiated.

It is significant that Eugster's group [15] had recourse to an X-ray structure determination to establish *cis* and *trans* stereochemical assignments to the dihydrofuran ring in a 7-oxabicyclo[4.3.0]non-9-ene system. Without the four actinidols for further NMR analyses, an X-ray study of a crystalline derivative of the major actinidols may be the only way to confirm the stereochemical assignment.

The major synthetic actinidols gave identical EIMS to those of the grape and wine components, and these two isomers symmetrically enhanced the peak of the natural pair when co-chromatographed with extracts of grape juice steam distillates on a capillary GC column.

On the basis of the evidence presented for the synthetic actinidols, the natural grape derived compounds are thus assigned *trans* stereochemistry also, i.e. **1a**.

EXPERIMENTAL

Low resolution MS: 70 eV GC/MS on a quadrupole instrument, scanning from m/z 35 to 350/sec with computer processing

Table 1. NMR spectral data for the actinidols (**1a**) and acetate esters (**1b**)

	^{1}H NMR (90 MHz, $CDCl_3$)						^{13}C NMR (22.49 MHz, $CDCl_3$)	^{1}H NMR (90 MHz, $CDCl_3$)						^{13}C NMR (22.49 MHz, $CDCl_3$)
	Actinidol R_t 1200		Actinidol R_t 1200 plus Eu(fod)$_3$		Acetate R_t 1264		Actinidol R_t 1200	Actinidol R_t 1211		Actinidol R_t 1211 plus Eu(fod)$_3$		Acetate R_t 1282		Actinidol R_t 1211
Position No.§	δ (ppm)	Multiplicity J(Hz)	δ (ppm)	Δ*	δ (ppm)	Multiplicity J(Hz)	δ (ppm)‡	δ (ppm)	Multiplicity J(Hz)	δ (ppm)	Δ*	δ (ppm)	Multiplicity J(Hz)	δ(ppm)‡
1	—	—	—	—	—	—	154.0	—	—	—	—	—	—	154.5
2	—	—	—	—	—	—	34.1	—	—	—	—	—	—	34.2
3	1.99	*d, d*; 3.2, 1.4	2.29	0.30	1.95	*m*,†	43.3	1.99	*d, d*; 3.2, 1.4	2.10	0.11	1.97	†	43.3
4	5.54	*d, t*; 10.0, 3.2	5.76	0.22	5.50	*d, d, d*; 10, 3.9, 3.0	132.5	5.57	*d, t*; 10.0, 3.2	5.58	0.01	5.50	*d, d, d*; 10.0, 3.8, 3.1	132.1
5	5.76	*d*(*br*); 10.0	6.89	1.13	5.76	*d*(*br*); 10.0	126.3	5.77	*d*(*br*); 10.0	5.77	0.00	5.76	*d*(*br*); 10.0	126.7
6	—	—	—	—	—	—	86.3	—	—	—	—	—	—	86.0
8	4.58	*d, d*; 4.8, 1.0	6.56	1.98	4.8–5.0	†‖	86.6	4.73	*d, d*; 3.1, 1.2	5.55	0.82	4.8–5.0	†‖	86.3
9	5.28	*s*(*br*); $W^{1/2}$ 2.4	6.07	0.79	5.28	*s*(*br*); $W^{1/2}$ 2.4	117.6	5.35	*s*(*br*); $W^{1/2}$ 2.5	5.66	0.31	5.23	*s*(*br*); $W^{1/2}$ 2.5	115.7
10	3.58	*d, q*; 6.2, 4.8	5.08	1.50	4.8–5.0	†	69.9	3.78	*d, q*; 6.6, 3.1	4.10	0.32	4.8–5.0	†	68.5
11	1.16	*d*; 6.2	1.60	0.44	1.13	*d*; 6.2	19.1	1.13	*d*; 6.6	1.54	0.41	1.08	*d, t*; 7.0	17.4
12	1.20	*s*	1.57	0.37	1.19	*s*	26.3	1.21	*s*	1.34	0.13	1.20	*s*	26.2
13	1.13	*s*	1.48	0.35	1.13	*s*	26.7	1.13	*s*	1.22	0.09	1.13	*s*	26.6
14	1.43	*s*	2.16	0.73	1.41	*s*	28.7	1.43	*s*	1.56	0.13	1.43	*s*	28.6
$COCH_3$	—	—	—	—	2.00	*s*	—	—	—	—	—	2.03	*s*	—

*Difference in chemical shift after Eu(fod)$_3$ addition. The absolute values of the differences cannot be compared for the two isomers because the spectra were taken with unequal concentrations of actinidols and with sequential addition of shift reagent.

†Obscured by overlapping signals.

‡Assignments were also made from published data [15] in analogous systems.

§See numbering in structure (**1**).

‖In benzene-d_6 this signal was not obscured and the NOE experiments were run in this solvent.

of output data; high resolution MS: direct probe insertion on a magnetic sector machine. GLC: FID and GC/MS with total ion current monitoring, SP1000 glass SCOT column (95 m × 0.5 mm), 50° for 10 min then to 180° at 1°/min, held at 180° for 20 min, injector and FID temps 225°, carrier gas He 2.7 ml/min. R_t's are those of Van Den Dool and Kratz [16]. Fourier transform NMR: $CDCl_3$ 90 MHz for ^1H and 22.49 MHz for ^{13}C; LC: as Still *et al.* [17]; TLC: silica gel 60 (1 mm) with Et_2O–pentane (1:3) or CH_2Cl_2. Anisaldehyde in HOAc–H_2SO_4 (1:100:2) for visualisation.

Hydrolysis of triols. The triols **3a** [6] or **4** [Strauss, C. R., Dimitriadis, E., Wilson, B. and Williams, P. J., in preparation] (500 mg) in pH 3 buffer (50 ml) (2% aq. tartaric acid adjusted with NaOH) were heated under reflux on a steam bath for 30 min. After cooling, the cloudy soln was extracted with Et_2O (2 × 30 ml). The hydrolysis and extraction were repeated seven times so that no further volatiles were yielded. The combined Et_2O extracts were dried ($MgSO_4$), concentrated by distillation of solvent through a fractionating column to give a fragrant yellow oil (*ca* 500 mg). LC of this with Et_2O–pentane (1:4) gave a fraction containing the actinidols **1a** and **2a** together with ketones (**5a**) (*ca* 200 mg).

Actinidyl acetates. The above actinidol–ketone fraction was acetylated and the mixture (220 mg) separated by LC, eluting with pentane–CH_2Cl_2 (1:1), through to CH_2Cl_2 alone. This gave the keto acetates **5b** (123 mg), TLC (CH_2Cl_2) R_f 0.45, and the actinidyl acetates **1b** and **2b** (82 mg), TLC (CH_2Cl_2) R_f 0.3. Further LC of the actinidyl acetates with Et_2O–pentane (1:4) was necessary to separate the major isomeric pair. Thus a high R_f (0.4) fraction (26 mg) and a low R_f (0.3) fraction (42 mg) were obtained.

The high R_f fraction was a colourless oil with a camphoraceous odour. GC R_t 1264; IR ν_{max} cm^{-1}: 1740, 1660, 1645, 1070, 840, 790, 720; MS *m/z* (rel. int.): 190 (6) $[M - HOAc]^+$, 175 (28), 163 (80), 157 (1), 149 (3), 147 (3), 145 (13), 135 (7), 133 (6), 131 (3), 130 (3), 121 (13), 119 (7), 107 (8), 105 (12), 95 (3), 93 (3), 91 (13), 81 (7), 79 (8), 77 (9), 69 (7), 65 (3), 55 (8), 43 (100).

The low R_f fraction contained predominantly one isomeric acetate, GC R_t 1282, with a trace amount of the two minor isomers, GC R_t 1330 and 1335. The IR characteristics of this fraction were almost identical with those of the high R_f fraction and the MS of the R_t 1264 and 1282 isomers showed only small differences in relative intensities. The minor isomers, R_t 1330 and 1335 each showed similar MS, i.e. *m/z* (rel. int.) 175 (11), 163 (35), 145 (7), 133 (7), 121 (9), 119 (9), 107 (11), 105 (14), 103 (6), 95 (8), 93 (9), 91 (16), 81 (15), 79 (8), 77 (12), 73 (17), 71 (7), 69 (16), 67 (6), 60 (18), 57 (12), 56 (8), 55 (23), 43 (100).

Actinidols. Hydrolysis of the high R_f acetate fraction with 5% KOH in MeOH gave, after isolation, a colourless oil with a camphoraceous odour. GC R_t 1200; IR ν_{max} cm^{-1}: 3450, 1660, 1640, 1080, 840, 795, 720 MS *m/z* (rel. int.): 193 (1), 164 (15), 163 (100), 149 (12), 145 (15), 135 (5), 133 (4), 131 (5), 121 (20), 119 (7), 107 (15), 105 (17), 93 (15), 91 (16), 81 (10), 79 (12), 77 (13), 69 (10), 65 (5), 57 (5), 55 (12), 53 (5), 45 (20), 43 (80). [Found: $C_{13}H_{20}O_2$–C_2H_5O; *m/z* 163.1119. Calc. for $C_{11}H_{15}O$; *m/z* 163.1123.]

Similarly, the low R_f acetate fraction gave on saponification the second major actinidol, GC R_t 1211, together with trace amounts of the minor isomers, GC R_t 1256 and 1332. IR ν_{max} cm^{-1}: 3440, 1660, 1640, 1075, 840, 795, 720. MS of the two major actinidols, GC R_t 1200 and 1211, showed only minor differences in intensities. The minor isomers, GC R_t 1256, showed MS *m/z* (rel. int.): 208 (0.1) $[M]^+$, 193 (1) 175 (1) 164 (16), 163 (100) 149 (8), 145 (14), 135 (6), 133 (5), 131 (5), 121 (20), 119 (11), 107 (10), 105 (17), 93 (15), 91 (19), 83 (9), 79 (10), 77 (13), 69 (10), 55 (12), 45 (16), 43 (65), 41 (19).

The actinidol isomer, GC R_t 1332, showed MS similar to that of the R_t 1256 isomer except for the absence of a molecular ion.

Mitsunobu inversion experiment [10]. To a stirred mixture of diethylazodicarboxylate (2 mg) and benzoic acid (2 mg) in Et_2O (1 ml) was added a mixture of actinidol, GC R_t 1200, (*ca* 3 mg) and triphenylphosphine (3 mg) in Et_2O (1 ml). After 2 hr, TLC (Et_2O–pentane) showed that the benzoate formed in the reaction had a different R_f to that of the benzoate from actinidol, GC R_t 1200, formed by direct esterification using $(PhCO)_2O$–pyridine. However, the R_f of the Mitsunobu benzoate was identical to that of the benzoate formed from actinidol, GC R_t 1211, directly with $(PhCO)_2O$–pyridine.

The benzoate formed from the Mitsunobu reaction of actinidol GC R_t 1200 was purified by LC and saponified (KOH–MeOH), to give the actinidol GC R_t 1211 and the product enhanced the peak of this isomer on co-GLC.

Similarly the actinidol, GC R_t 1211, was converted, via the inversion reaction, to a benzoate which corresponded by TLC with that formed by direct esterification of the GC R_t 1200 actinidol isomer. Saponification of this inverted acetate gave the shortest R_t actinidol.

Acknowledgements—We thank The Reserve Bank of Australia Rural Credits Development Fund, for supporting one of us (E.D.) and Dr. G. Jones, J. P. Quirk NMR facility, Waite Agricultural Research Institute, for recording the NMR spectra and for valuable discussion.

REFERENCES

1. Masuda, M. and Nishimura, K. I. C. (1980) *J. Food Sci.* **45**, 396.
2. Williams, P. J., Strauss, C. R., Wilson, B. and Massy-Westropp, R. A. (1982) *J. Chromatogr.* **235**, 471.
3. Simpson, R. F., Strauss, C. R. and Williams, P. J. (1977) *Chem. Ind. (London)* 663.
4. Dimitriadis, E. and Williams, P. J. (1984) *Am. J. Enol. Vitic.* **35**, 66.
5. Sakan, T., Isoe, S. and Hyeon, S. B. (1967) *Tetrahedron Letters* 1623.
6. Isoe, S., Hyeon, S. B., Ichikawa, H., Katsumura, S. and Sakan, T. (1968) *Tetrahedron Letters* 5561.
7. Naves, Y. R. (1971) *J. Soc. Cosmet. Chem.* **22**, 439.
8. Takazawa, O., Kogami, K. and Hayashi, K. (1983) *Chem. Letters* 63.
9. Uegaki, R., Fujimori, T., Kaneko, H., Kato, K. and Noguchi, M. (1979) *Agric. Biol. Chem.* **43**, 1149.
10. Mitsunobu, O. (1981) *Synthesis* 1.
11. Cadosch, H., Vögeli, U., Rüedi, P. and Eugster, C. H. (1978) *Helv. Chim. Acta* **61**, 783.
12. Cadosch, H., Vögeli, U., Rüedi, P. and Eugster, C. H. (1978) *Helv. Chim. Acta* **61**, 1511.
13. Alder, A. P., Wolf, H. R. and Jeger, O. (1978) *Helv. Chim. Acta* **61**, 2681.
14. Bischofberger, N., Frei, B. and Jeger, O. (1983) *Helv. Chim. Acta* **66**, 1638.
15. Acemoglu, M., Prewo, R., Bieri, J. H. and Eugster, C. H. (1984) *Helv. Chim. Acta* **67**, 175.
16. Van Den Dool, H. and Kratz, P. D. (1963) *J. Chromatogr.* **11**, 463.
17. Still, W. C., Kahn, M. and Mitra, A. (1978) *J. Org. Chem.* **43**, 2923.