(4H, *m*, 2× $-CH_2-$), 3.00–3.25 (2H, *m*, 2× –CH–), 3.58, 3.60, 3.61 (each 3H, *s*, $-CO_2Me$); ^{13}C NMR (50.32 MHz, $CDCl_3$): 13.99, 22.60, 28.70, 29.30, 29.49, 29.51, 32.26, 42.90, 46.10, 51.71 (–OMe), 52.04 (–OMe), 172.08 (–CO–), 173.29 (–CO–), 174.17 (–CO–); MS *m/z* (rel. int.): 414 $[M]^+$ (17), 383 (68), 382 (58), 350 (40), 322 (49), 295 (25), 269 (63), 242 (71), 228 (71), 218 (85), 210 (61), 196 (58), 186 (90), 154 (73), 146 (100), 126 (85), 114 (98), 98 (61), 84 (84).

(+)-*Norrangiformic acid* (**3**). Hydrolysis of **1** with KOH + MeOH gave **3**: mp 105–108° (MeOH); $[\alpha]_D^{24}$ +7.5° (MeOH; *c* 1.87); $C_{20}H_{36}O_6$ (372.49). IR ν_{max}^{KBr} cm^{-1}: 670, 716, 734, 764, 792, 848, 882, 924, 1020, 1126, 1176, 1212, 1230, 1270, 1402, 1470, 1670 ($-CO_2H$), 1700 ($-CO_2H$), 2850, 2930, 3150 ($-CO_2H$); MS *m/z* (rel. int.): 354 $[M-H_2O]^+$ (54), 336 (73), 318 (38), 308 (77), 290 (75), 280 (84), 264 (70), 252 (57), 238 (49), 220 (61), 214 (67), 210 (61), 200 (81), 196 (87), 186 (80), 182 (98), 168 (96), 154 (91), 140 (95), 126 (86), 112 (93), 98 (100), 84 (95).

(+)-*Isorangiformic acid anhydride* (**4**). Heating **1** (0.3 g) with AcCl (7 ml) under reflux (2 hr), removal of excess AcCl (*in vacuo*) and crystallization of the residue from *n*-hexane gave **4**: needles, mp 92–94°; $[\alpha]_D^{24}$ + 13.4° ($CHCl_3$; *c* 1.74); $C_{21}H_{36}O_5$ (368.50). IR ν_{max}^{KBr} cm^{-1}: 716, 742, 784, 818, 840, 866, 892, 928, 940, 964, 1000, 1014, 1040, 1094, 1190, 1210, 1240, 1280, 1300, 1338, 1380, 1436, 1464, 1702 ($-CO_2Me$), 1758, 1790 (anhydride –CO–), 2870, 2950.

The NaOH fraction of the Et_2O extract gave, after acidification and crystallization from $CHCl_3$–MeOH, (+)-usnic acid (3.5 g, 0.44%): mp 198–200°; $[\alpha]_D^{24}$ + 492° ($CHCl_3$; *c* 0.50).

The neutral part of the Et_2O extract yielded after chromatography on SiO_2 and crystallization from $CHCl_3$–MeOH, zeorin (0.21 g, 0.026%) as double pyramids, mp 230–234°.

Acknowledgement—I thank Professor Dr. J. Poelt, Botanical Institute, University of Graz, Austria, for the identification of *L. stenotropa*.

REFERENCES

1. Huneck, S. ad Ahti, T. (1982) *Pharmazie* (in press).
2. Åkermark, B. (1967) *Acta Chem. Scand.* **21**, 589.
3. Nakanishi, K. (1962) in *Infra Red Absorption Spectroscopy* p. 45. Holden-Day, San Francisco.

Phytochemistry, Vol. 21, No. 9, pp. 2408–2410, 1982.
Printed in Great Britain.

0031-9422/82/092408-03$03.00/0

TERPENES IN *PISTACIA* PLANTS: A POSSIBLE DEFENCE ROLE FOR MONOTERPENES AGAINST GALL-FORMING APHIDS

PIETRO MONACO, LUCIO PREVITERA and LORENZO MANGONI

Institute of Organic and Biological Chemistry of the University, Via Mezzocannone 16, 80134 Napoli, Italy

(*Revised received* 22 *January* 1982)

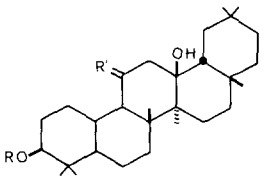
Key Word Index—*Pistacia*; Anacardiaceae; aphids; triterpenes; monoterpenes.

Abstract—A re-investigation of *Pistacia vera* from southern Italy afforded, in addition to known compounds, two new monoterpenes (+)-9,10-cyclopropylterpinen-4-ol and (+)-9,10-cyclopropyl-terpin-2,4-diol and a novel triterpene triol 3β,11α,13β-trihydroxyoleanane. The possible defence role of monoterpenes against gall-forming aphids is discussed briefly.

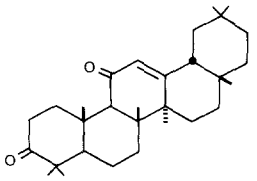
INTRODUCTION

The genus *Pistacia* includes many species widely distributed in the Mediterranean and Middle East areas which are often infected by gall-forming insects. A previous investigation of resins exuded from galls and trunks of several species [1] showed no significant differences in chemical composition.

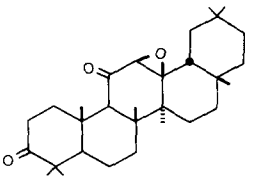
Some of us recently reported [2] a comparative study on oleoresins from trunks of *Pistacia atlantica* plants originating in Israel and Iran. Both were found to consist of euphane, dammarane and oleanane triterpenes, the only relevent difference being the presence in the oleoresin from Iranian specimens of two pinane monoterpenes. Interestingly, *P. atlantica* from Israel produces galls due to the aphid *Slavum wertheimae* (genus *Forda*) whereas the same species from Iran is gall-free although this insect lives in Iran and infects other *Pistacia* species growing in the same area [3]. We have now examined oleoresins from trunks of uninfected plants of *P. vera* from



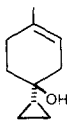
1a R = H R' = H, α OH
1b R = H R' = H, β OH
1c R = Ac R' = H, α Ac
1d R = Ac R' = H, β OH



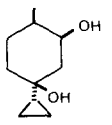
2



3



4



5

Italy, having already studied [4] plants of the same species from Iran infected by *Pemphigus utricularius* (genus *Forda*).*

RESULTS AND DISCUSSION

The resin was collected during the summer and was worked up in the usual way to afford, after repeated chromatography, besides the triterpenes already isolated from the infected plants, a new pentacyclic tripterpene **1a** and five monoterpenes.

Compound **1a**, after crystallization from hexane, had mp 208–210°, $[\alpha]_D + 5.5°$, ν_{max} 3640 cm^{-1} and molecular formula $C_{30}H_{52}O_3$. The NMR spectra closely resembled that of β-amyrin except for the three methyl signal position, an additional signal at δ 3.71 (1H, $W_{1/2}$ 25 Hz) and the absence of vinyl protons. Acetylation of **1a** under mild conditions afforded the diacetate **1c** showing residual hydroxyl absorption in the IR spectrum. NMR data showed two signals at δ 4.48 and 5.00, each due to an axial proton geminal with an acetoxyl group. On this basis, structure 3β,11α,13β-trihydroxyoleanane can be suggested for **1a**.

Such a structure as proved through a partial synthesis starting from 11-oxo-β-amyrenone (**2**)[5], which, by reaction with *m*-chloroperbenzoic acid, was converted into the 12β-epoxy compound **3**. The formation of only the β-epoxide was expected on the basis of molecular models of **2** and from the results of Barton *et al.* on β-amyrenone oxidation[6]. $LiAlH_4$ reduction of **3** gave two C-11 epimeric triols which were separated by CC. The minor component was identical in every respect with natural **1a**. The assigned stereochemistry at C-11 was confirmed by acetylation: synthetic **1a** gave a diacetate identical with **1c**; by contrast the epimeric triol **1b** gave exclusively monoacetate **1d** owing to the intramolecular hydrogen bond between β-hydroxyl groups at C-11 and C-13 and to the 1,3 diaxial interaction between the C-11β hydroxyl group and the C-25 and C-26 methyl groups. As far as monoterpenes are concerned, three were recognized to be (+)-*trans*-verbenol, (−)-pinocarveol and terpinolene; compounds **4** and **5** were new and were assigned the structures (+)-9,10-cyclopropylterpinen-4-ol and (+)-9,10-cyclopropyl-terpin-2,4-diol respectively on the basis of their physical features and by comparison with synthetic samples[7].

Do monoterpenes represent a defence strategy against aphids in the genus *Pistacia*? Although our knowledge of plant–insect interactions is far from complete, it is clear that many monoterpenes have insect-repellent and attractant properties[8]. Since gall-free specimens of *P. atlantica* as well as of *P. vera* have been found to contain monoterpenes, it is possible that these compounds play a specific repellent role against aphids and are responsible for the lack of galls. Such a hypothesis, which requires further support, may be important agriculturally because these insects infect other families such as the Gramineae, Leguminosae and Solanaceae[9].

EXPERIMENTAL

NMR spectra were performed at the Centro di Metodologie Chimico Fisiche of the University in $CDCl_3$ solns using TMS as int. standard. General procedures of extraction and separation of acid and neutral fractions have been described[7]. Known compounds were identified by comparing their IR and ^1H NMR spectra with those of authentic material.

3β,11α,13β-Trihydroxyoleanane (**1a**). A fraction from the general chromatography (400 mg; petrol–Et_2O, 7:3) was crystallized from hexane to give pure **1a**: mp 208–210°; $[\alpha]_D + 5.5°$ (c1.1 in $CHCl_3$); MW 460 (MS); IR ν_{max} 3640 cm^{-1}; ^1H NMR: δ 0.78 (*s*, 3H), 0.87 (*s*, 6H), 0.91 (*s*, 3H), 0.96 (*s*, 3H), 0.97 (*s*, 3H), 0.99 (*s*, 3H), 1.06 (*s*, 3H), 3.23 (*m*, 1H, $W_{1/2}$ 22.5 Hz) and 3.71 (*m*, 1H, $W_{1/2}$ 25 Hz).

3β,11α-Diacetoxy-oleanan-13β-ol (**1c**). Pure **1a** (30 mg) was treated with Ac_2O in pyridine overnight to afford diacetate **1c** mp 194–195° (from MeOH); $[\alpha]_D + 5.0°$ (c0.9 in $CHCl_3$); IR ν_{max} 3640, 1730 cm^{-1}; ^1H NMR: δ 1.97 (*s*, 3H, MeCOO– at C-11), 2.00 (*s*, 3H, MeCOO–at C-3), 4.48 (*m*, 1H, H-3α), 5.00 (*m*, 1H, H-11β).

Synthesis of **1a**. 11-Oxo-β-amyrenone (**2**) mp 237–240° (120 mg), prepared according to ref. [5] from β-amyrin, in dry CH_2Cl_2 (1.5 ml) was added at 0° with *m*-Cl-perbenzoic acid (80 mg). After 1 hr with stirring, the acid was filtered off and solution was washed with 2 N thiosulfate and satd. Na_2CO_3. CC of the crude reaction mixture gave (C_6H_6–Et_2O, 19:1) β-epoxy compound **3** (100 mg) mp 213–218° (from hexane–C_6H_6, 4:1); $[\alpha]_D$ −11.4° (c1.1 in $CHCl_3$). $LiAlH_4$ reduction of **3** (90 mg) resulted in a mixture of C-11 epimeric triols which was directly chromatographed on Si gel to give **1b** (53 mg; petrol–Et_2O, 4:1) mp 223–225° (from hexane); $[\alpha]_D + 37.8°$ (c0.6 in $CHCl_3$); IR ν_{max} 3640, 3520 cm^{-1}; ^1H NMR: δ 3.22 (*m*, 1H, H-3α), 3.97 (*m*, 1H, $W_{1/2}$ 8.8 Hz, H-11α); further elution with petrol–Et_2O (7:3) gave a triol, mp 208–210° (27 mg) identical with natural **1a**.

Acetylation of **1a** *and* **1b**. Acetylation procedure has already been described: synthetic **1a** gave a diacetate mp 194–195° identical with **1c**. Triol **1b** gave monoacetate **1d** mp 270–275° (from hexane); $[\alpha]_D + 45.0°$ (c1.0 in $CHCl_3$); IR ν_{max} 3640, 3520, 1730 cm^{-1}; ^1H NMR: δ 2.00 (*s*, 3H, MeCOO– at C-3), 3.95 (*m*, 1H, H-11α), 4.17 (*m*, 1H, H-3α).

**P. utricularius* is present in the Mediterranean area and in Italy forms galls on the leaves of *P. terebinthus*.

REFERENCES

1. Caputo, R., Mangoni, L., Monaco, P. and Palumbo, G. (1978) *Phytochemistry* **18**, 896.
2. Aynechi, Y., Caputo, R., Mangoni, L., Monaco, P. and Palumbo, G. (1979) Work presented at 11th Organic Chemistry Meeting of Italian Chemical Society, Sorrento.
3. Bodenheimer, F. S. and Swirski, E. (1957) *The Aphidoidea of the Middle East*, The Weizmann Science Press of Israel.
4. Caputo, R., Mangoni, L., Monaco, P., Palumbo, G., Aynehchi, Y. and Bagher, M. (1978) *Phytochemistry* **17**, 815.
5. Taylor, D. A. H. (1967) *J. Chem. Soc.* 490.
6. Barton, D. H. R., Boar, R. B., Knight, D. C. and McGhie, J. F. (1970) *J. Chem. Soc. C*, 678.
7. Mangoni, L., Monaco, P. and Previtera, L. (1982) *Phytochemistry* **21**, 811.
8. Harborne, J. B. (1977) *Introduction to Ecological Biochemistry*, p. 167. Academic Press, New York.
9. Wertheim, G. (1954) *Trans. R. Ent. Soc. Lond.* **105**, 79.

Phytochemistry, Vol. 21, No. 9, pp. 2410–2411, 1982
Printed in Great Britain.

0031-9422/82/092410-02$03.00/0

A HALOGENATED CHAMIGRANE EPOXIDE AND SIX RELATED HALOGEN-CONTAINING SESQUITERPENES FROM THE RED ALGA *LAURENCIA OKAMURAI*

MAKOTO OJIKA, YOSHIKAZU SHIZURI and KIYOYUKI YAMADA

Department of Chemistry, Faculty of Science, Nagoya University, Chikusa, Nagoya 464, Japan

(*Revised received* 1 *March* 1982)

Key Word Index—*Laurencia okamurai*; Rhodomelaceae; red alga; sesquiterpenes; halogenated chamigranes; 4, 10-dibromo-3-chloro-7α, 8α-epoxy-α-chamigrene.

Abstract—From the red alga *Laurencia okamurai* a new chamigrane epoxide and six known halogenated chamigranes were isolated. The structure of the new epoxide was established by spectral and chemical means.

INTRODUCTION

Previous investigation of the red alga *Laurencia okamurai* (Rhodomelaceae, Rhodophyta) has revealed that the aromatic sesquiterpenes of the laurane- and cuparane-types are characteristic metabolites of the alga, fifteen such compounds having been isolated [1–3]. Johnstonol was a sole member of the chamigrane-type sesquiterpene that was previously isolated in a minute amount from *L. okamurai* [1, 4]. We now describe the isolation from this alga of a new halogenated chamigrane (**1**) and six halogenated chamigranes, 4,10-dibromo-3-chloro-α-chamigrene (**2**) [5, 6], 4,10-dibromo-3-chloro-9-hydroxy-α-chamigrene (**3**) [7], prepacifenol epoxide (**4**) [8], prepacifenol (**5**) [9], 1-deoxyprepacifenol (**6**) [10] and nidificene (**7**) [11], together with aromatic sesquiterpenes of the laurane-type such as aplysin [1, 3, 12], debromoaplysin [1, 3, 12], laurinterol [2, 3, 13], and isolaurinterol [2, 3, 13].

RESULTS

The fresh alga was extracted with acetone and the resulting extract was further extracted with benzene–ethyl acetate. The oily extract was separated by CC on Si gel and prep. TLC (Si gel) to give the new compound (**1**), **2**, **3**, **6**, **7**, johnstonol (**8**) [4], and pacifenol (**9**) [14].

Since **4** and **5** were found to be transformed partially or completely into **8** and **9**, respectively, during CC and TLC, the crude extract of the fresh alga was subjected to separation employing prep. HPLC with a column of the reversed phase [LiChrosorb RP-8 and RP-18; methanol–water (4:1)] to afford **4** and **5**. Johnstonol (**8**) and pacifenol (**9**) were not detected by analytical TLC of the crude extract, and therefore **8** and **9**, obtained after chromatographic separation, must be artifacts.

The structural elucidation of the new compound (**1**) is as follows. High resolution mass spectral data of **1** established a molecular formula of $C_{15}H_{23}OBr_2Cl$. ^1H NMR spectral data of **1** was similar to but not identical with 4,10-dibromo-3-chloro-7β,8β-epoxy-α-chamigrene (**10**) [5], isolated previously from the same algal genus. Based on the spectral data, the new compound was deduced to have structure **1**. This was confirmed by direct comparison with a sample of **1** obtained by oxidation of **2** with *m*-chloroperbenzoic acid. Synthesis of **1** from **2** was reported previously [5], but this is the first time that **1** has been isolated as a natural product.