BIOLOGICAL ASSAYS.—With the aid of a Hamilton 5-μl microsyringe, graded amounts (0.5-2.5 μl) of oil of *C. anisata* were applied topically to the ventral region of third nymphal instars of the variegated grasshopper *Z. variegatus*. The insects were collected from the field at first nymphal instar and were raised in the laboratory on the leaves of Cassava, *Manihot esculenta* (5). Inasmuch as each nymphal stadium of the grasshopper lasted 2-3 weeks, the insects were, thus, well-conditioned in the laboratory before the biological screening. Control insects were untreated. There were 10 insects for each concentration, and each treatment was replicated 3 times. Insects for each treatment were placed inside half-liter kilner jars with screen covers (6). Mortality data were corrected by Abbott's formula (7). The probit mortality against log concentration regression line was computed following methods employed by W.H.O. for malaria vector control (8).

After 24 h, LD_{50} for the crude oil was 3780 mg/kg while that of estragole was 2430 mg/kg. Toxic symptoms observed included restlessness among treated insects, tucking of heads ventrally by extending the dorsal cervix, and the antennae being continuously cleaned by the forelegs. Mortality occurred without kicking or jerking of legs.

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LITERATURE CITED

1. Th. M. Meijer, *Rec. trav. chim.*, **66**, 395 (1947).
2. D. Novak, *Proc. Int. Congr. Acarol.*, 3rd, 685 (1971).
3. F.I. Opute, *J. Expt. Bot.*, **26**, 619 (1975).
4. R.F. Heck, *J. Am. Chem. Soc.*, **90**, 5531 (1968).
5. J.I. Olaifa, *Insect Sci. Applic.*, **7**, 135 (1986).
6. J.I. Olaifa, W.O. Erhun, and A.E. Akingbohungbe, *Insect Sci. Applic.*, in press.
7. W.S. Abbott, *J. Econ. Ent.*, **18**, 265 (1925).
8. S. Swaroop, A.B. Gilroy, and K. Vemura, in: "Statistical Methods in Malaria Eradication," W.H.O. Geneva, 1966, pp. 117-129.

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VOLATILES FROM THE ROOT OF *DIPLOTAENIA CACHRYDIFOLIA*, THE FIRST NATURAL SOURCE OF 6-CAMPHENONE

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A phytochemical survey of *Diplotaenia cachrydifolia* Boiss. (Umbelliferae) showed that the plant contained a volatile fraction in the root in addition to the essential oil of the fruit. The plant has also been found to contain the furanocoumarins xanthotoxin, bergapten, and isopimpinellin in the fruit and root (1), the dihydropyranocoumarins jatamansin (leaf, fruit, and root) and jatamansinol (root) (2), and the dihydrofuranocoumarins columbianetin and columbianadin (root) (3).

The plant grows at elevations of up to 2,600 m in the Kandavan Chalus Valley 100 km north of Tehran and also in other regions of Iran and in Turkey (4-7).

Only two species of *Diplotaenia* have been recorded to date. *Diplotaenia damavendica*, which occurs at elevations of up to 3,000 m around Lake Tar 120 km north of Tehran, is reported to contain xanthotoxin and angelicin, the latter causing photosensitization of the skin (8).

This is the first reported isolation and analysis of volatiles from the root of this species. 6-Camphenone (3,3-dimethyl-2-methylenebicyclo[2.2.1]-heptan-6-one) [**1**], the major constituent (43.0%), is the ketone of nojigiku alcohol (6-camphenol) and was first synthesized in 1974 (9). The oil also contained the biosynthetically related compounds 6-camphenol (nojigiku alcohol, 1.2%) and nojigiku acetate. These constituents have not previously been found in the volatile oil derived from the fruits of this species. Individual identification of the peaks is listed in Table 1.

TABLE 1. Composition of the Essential Oil of *Diplotaenia cachrydifolia* Root[a]

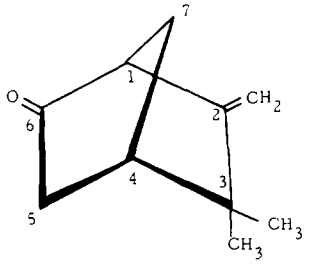
	Component	RI BP-1	RI BP-20	Relative Conc. (%)	Mode of Identification
1.	α-pinene	937	1030	1.7	r,ms(11)
2.	sabinene	967	1123	1.0	r,ms(12)
3.	β-pinene	980	1120	0.3	r,ms(11)
4.	myrcene	982	1165	t	r,ms(11)
5.	3-carene	1008	1158	t	r,ms(11)
6.	*cis*-ocimene	1025	1234	0.1	r,ms(13)
7.	limonene	1026	1205	0.4	r,ms(11)
8.	*trans*-ocimene	1038	1250	0.7	r,ms(13)
9.	γ-terpinene	1053	1253	0.9	r,ms(11)
10.	4-decyne	1060	1436	1.5	ms(13)
11.	6-camphenone	1085	1492	43.0	ms,ir(14-17)
12.	6-camphenol (nojigiku alcohol)	1102	1541	1.2	ms,ir(14-17)
13.	camphor	1132	1508	3.1	r,ms(18)
14.	lavandulol	1152	1679	6.0	ms(19,20)
15.	4-terpineol	1168	1613	3.4	r,ms(12)
16.	*cis*-verbenol	1169	2095	1.0	ms(21)
17.	safranal	1170	1729	1.1	ms(22)
18.	myrtenal	1174	1693	0.4	ms(21)
19.	dihydrocarveol	1184	1714	0.7	ms(21)
20.	*cis*-carveol	1205	1780	1.7	ms(21)
21.	pulegone	1217	1690	2.1	r,ms(21)
22.	carvone	1220	1729	0.7	r,ms(20)
23.	nojigiku acetate	1228	1547	2.8	ms(14)
24.	linalyl acetate	1238	1566	0.6	r,ms(22)
25.	geranial	1244	1735	0.5	r,ms(18)
26.	unidentified	1259	1727	0.7	[M]+ at *m/z* 134
27.	bornyl acetate	1271	1580	2.1	ms(23)
28.	unidentified	1285	1770	1.4	[M]+ at *m/z* 150
29.	unidentified	1301	1776	0.6	[M]+ at *m/z* 136
30.	bergamotene	1454	1646	1.4	ms(24)
31.	unidentified	1504	1808	0.8	[M]+ at *m/z* 202
32.	elemicin	1530	2239	0.3	ms(13,25,26)
33.	unidentified	1568	1958	1.2	[M]+ at *m/z* 204
34.	unidentified	1585	1980	1.7	[M]+ at *m/z* 204
35.	unidentified	1592	2175	0.7	[M]+ at *m/z* 222
36.	unidentified	1593	1773	2.3	r, [M]+ at *m/z* 148 (base peak *m/z* 133)
37.	unidentified	1595	2190	0.7	[M]+ at *m/z* 220
38.	iso-elemicin	1619	2480	0.7	ms(13,26)
39.	guaiene	1635	2360	2.9	r,ms(24)
40.	unidentified	1638	2380	1.2	[M]+ at *m/z* 222
41.	unidentified	1682	2401	0.8	[M]+ at *m/z* 222
42.	unidentified	1848	2685	1.0	[M]+ at *m/z* 272

[a]Key: t=trace,<0.1%.
r=by comparison with authentic reference compounds.
ir=identification confirmed by infra-red spectroscopy.
ms=identification confirmed by mass spectrometry.

EXPERIMENTAL

PLANT MATERIAL.—Plants were collected in Iran at the ripe-fruit stage in September 1985, and authenticated at the Herbarium of The Royal Botanic Gardens, Kew, where a voucher specimen is deposited.

EXTRACTION AND FRACTIONATION.—Dried, powdered root (1 kg) was steam distilled with a



1 6-camphenone

Clevenger apparatus (10) and yielded 1 ml (0.1%) of a yellow, heavy, essential oil. The oil was dried over Na_2SO_4 and stored in a sealed container. The oil was fractionated over Si gel (20 g), eluted with hexane (100 ml) followed by $CHCl_3$ (100 ml) and by EtOAc (100 ml) to give six fractions. The oil and each of the fractions were analyzed by capillary gc.

GAS CHROMATOGRAPHY.—Capillary glc was carried out on both BP-1 (Scientific Glass Engineering, 50QC3/BP1-1.0) and BP-20 (Scientific Glass Engineering, 25QC3/BP20-0.5) columns using a Perkin-Elmer Series 8320 chromatograph fitted with a flame ionization detector. Temperature programming was performed, the BP-1 column from 70-200° at 2° min. and the BP-20 column from 60-180° at 2° min. Gc/ms was carried out on an SE 30 capillary column. Peaks were identified by means of Kovats retention indices and mass spectra. Authentic reference compounds were used for comparison wherever possible. Chromatograms were quantified by peak area normalization on the BP-1 column.

The oil exhibited the following physical properties: $d^{25}=0.941$; $[\alpha]^{25}D=-164$ to $-176°$ (2% in $CHCl_3$).

ISOLATION OF 6-CAMPHENONE.—Isolation was carried out by column fractionation and identified by comparison with published data (9): mp=46-47° (9); $[\alpha]^{25}D=+33.5°$ (2% in $CHCl_3$) (9, 14); ir λ max ($CHCl_3$) at 2950, 1740, 1670, 1460, 1378, 1250, 1050, 900 cm^{-1} (14); ms *m/z* (% relative intensity) 151 ($M+1^+$, 1), 150 (M^+, 5), 135 (1), 117 (1), 107 (28), 91 (36), 79 (25), 77 (35), 67 (5), 65 (18), 53 (23), 50 (25), 41 (100), 39 (71) (14).

LITERATURE CITED

1. K.J. Harkiss and M.H. Salehy Surmaghy, *Fitoterapia*, in press.
2. K.J. Harkiss and M.H. Salehy Surmaghy, *Fitoterapia*, in press.
3. K.J. Harkiss and M.H. Salehy Surmaghy, submitted for publication.
4. A. Parsa, "Flora de l'Iran," Vol. 2, 796, Ministry de l'Education, Tehran, 1943, p. 796.
5. V. Mozaffarian, "The Family of Umbelliferae in Iran, Keys and Distribution," Vol. 35, Research Institute of Forests and Rangelands, Tehran, 1983, p. 106.
6. E. Boissier, "Flora Orientalis," Vol. 3, Genevae et Basileae, 1872, p. 981.
7. P.H. Davis, "Flora of Turkey," Vol 4, Edinburgh University Press, 1972, p. 440.
8. Y. Aynehchi, "Pharmacognosy and Medicinal Plants of Iran," Vol. 1879, Tehran University Publications, 1986, p. 209.
9. A. Matsuo, Y. Uchio, M. Nakayama, Y. Matsubara, and S. Hayashi, *Tetrahedron Lett.*, **48**, 4219 (1974).
10. M.E. Crespo, J. Cabo, J. Jimenez, C. Navarro, and A. Zarzuelo, *J. Nat. Prod.*, **49**, 558 (1986).
11. A.F. Thomas and B. Willhalm, *Helv. Chim. Acta*, **47**, 475 (1964).
12. R. Ryhage and E. von Sydow, *Acta Chem. Scand.*, **17**, 2025 (1963).
13. "Eight Peak Index of Mass Spectra," Vol. 2. Mass Spectrometry Data Centre, AWRE, Aldermaston, United Kingdom, 1974.
14. Y. Uchio, *Bull. Chem. Soc. Japan*, **51**, 2342 (1978).
15. N. Darby, N. Lamb, and T. Money, *Can. J. Chem.*, **57**, 742 (1979).
16. M. Gaitonde, P.A. Vatakencharry, and D. Sukh, *Tetrahedron Lett.*, **30**, 2007 (1964).
17. M. Julia, D. Mansuy, and P. Detraz, *Tetrahedron Lett.*, **25**, 2141 (1976).
18. E. von Sydow, *Acta Chem. Scand.*, **18**, 1099 (1964).
19. E. von Sydow, *Acta Chem. Scand.*, **17**, 2504 (1963).
20. A.F. Thomas, B. Willholm, and J.H. Bowie, *J. Chem. Soc.*, **13**, 392 (1967).
21. A.A. Swigar and R.M. Silverstein, "Monoterpenes," Aldrich Chemical Company, Inc., Milwaukee, 1981.
22. S. Dev, A.P.S. Narula, and J.S. Yadav, "CRC Handbook of Terpenoids, vol. II," CRC Press, Inc., Boca Raton, 1981.

23. W. Jennings and T. Shibamoto, "Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography," Academic Press, London, 1980.
24. M.G. Moshonas and E.D. Lund, *Flav. Ind.*, 375 (1970).
25. A.M. Geisbrecht, N.C. Franca, and O.R. Gottlieb, *Phytochemistry*, **13**, 2285 (1974).
26. G.M. Sammy and W.W. Nawar, *Chem. Ind.*, 1279 (1968).

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JASPAMIDE FROM THE MARINE SPONGE *JASPIS JOHNSTONI*[1]

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Until now, a limited number of sponges of the genus *Jaspis* (Jaspidae) have been investigated for their content in secondary metabolites. Several bright yellow triterpenes derived from the rare malabaricane skeleton have been reported from Fijian (1) and Great Barrier Reef (2) collections of *Jaspis stellifera*. Very recently, jaspamide, a cyclic depsipeptide, has been isolated by two independent groups from an unspecified *Jaspis* species collected off the Fiji and Palau Islands (3,4).

Jaspamide exhibited potent insecticidal, antifungal, anthelminthic, and in vitro cytotoxic activities (3,4). In the course of our systematic search for toxic derivatives from marine sponges (5), we have observed that the CH_2Cl_2 extract of the New Guinean marine sponge *Jaspis johnstoni* Schmidt (syn. *Zapethea digonoxea*) is toxic for the freshwater fish *Lebistes reticulatus* (LD<5 mg/liter). Fractionation of this extract monitored by toxicity testing led to the isolation of a single derivative whose spectral properties were identical to those of jaspamide.

EXPERIMENTAL

BIOLOGICAL MATERIAL.—Specimens of *J. johnstoni* (550 g dry weight) were collected off Laing Island, Madang Province, Papua, New Guinea. The sponge has been authenticated by Dr. P.A. Thomas (India) and Dr. J. Vacelet (Marseille, France). A voucher specimen has been deposited in the Laboratory of Bio-organic Chemistry of the University of Brussels (no XIV.9).

EXTRACTION AND ISOLATION.—Sun-dried, ground sponges (350 g) were exhaustively extracted at room temperature with CH_2Cl_2 to give a residue (2.08 g) that was chromatographed on a Si gel column using increasing concentrations of Me_2CO in hexane. The fractions showing toxicity against the freshwater fish *L. reticulatus* (6) were pooled and chromatographed on a second Si gel column using increasing concentrations of Et_2O in CH_2Cl_2 to give jaspamide (54 mg) as a colorless solid homogenous on tlc and hplc [$C_{36}H_{45}N_4O_6$ Br by hrfabms; uv (MeOH) λ max at 220 (42,340), 275 (9,190), 289 nm (7,015); ir (film) characteristic bands at 3300, 1710, 1670, 1635, 1620, 1510, 830, 740 cm^{-1}].

Analysis of the $^1H/^1H$ and $^1H/^{13}C$ correlated 2D-nmr spectra (COSY and COLOC) and nOe experiments as well as microhydrolysis results (HCl 6N and CH_3SO_3H 4N) led to the conclusion that the compound is a depsipeptide containing a tripeptide moiety linked to a polyketide chain. At this stage of the work two papers (3,4) appeared describing the isolation of jaspamide from an unspecified *Jaspis* species. Comparison of the spectral data of our compound with those reported for jaspamide established the identity of the two compounds.

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