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CRISPOLIDE, AN UNUSUAL HYDROPEROXYSESQUITERPENE LACTONE FROM *TANACETUM VULGARE*

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Key Word Index—*Tanacetum vulgare* var. *crispum*; *T. vulgare* chemotypes; Compositae; sesquiterpene lactones; hydroperoxides; re-arranged germacranolides; crispolide; peroxyparthenolide; parthenolide.

Abstract—Crispolide, a hydroperoxysesquiterpene lactone with a modified germacrane skeleton, was isolated from the aerial parts of *Tanacetum vulgare* var. *crispum* and from two *T. vulgare* chemotypes. Its structure, 1β-hydroperoxy-5β-hydroxy-4,14-cyclogermacra-9,11-dien-6,12-olide, was elucidated by spectroscopic data and chemical reactions.

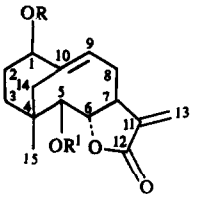
INTRODUCTION

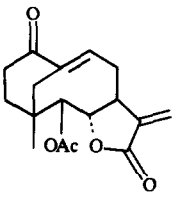
During a recent chemosystematic investigation of the mono- and sesquiterpene compounds of *Tanacetum vulgare* [1–3], it was found that chloroform extracts of *T. vulgare* var. *crispum* gave positive reactions for the presence of peroxides (liberation of iodine from ethanolic potassium iodide, blood-red colour with ferrous thiocyanate). These reactions were also given by some extracts of *T. vulgare* chemotypes. Examination of the constituents of *T. vulgare* var. *crispum* and of two chemotypes of *T. vulgare* giving positive tests for peroxides, resulted in the isolation of the hydroperoxysesquiterpene lactone **1**, which was named crispolide. The elucidation of the structure of this novel compound is reported in this paper.

RESULTS AND DISCUSSION

Crispolide (**1**, $C_{15}H_{20}O_5$) was isolated as a white amorphous powder giving positive chemical tests for peroxides. Its UV absorption at 210 nm (log ε = 4.06) and IR absorption bands at 1745 and 1650 cm^{-1} suggested the presence of an α,β-unsaturated γ-lactone. This assignment was further supported by the characteristic pair of proton signals at 6.26 (1H, *d*, $J = 3.3$ Hz) and 5.45 (1H, *d*, $J = 3.1$ Hz) and carbon signals at 169.5 (*s*), 140.2 (*s*) and 118.3 (*t*) (RO–CO–C=CH_2).

The presence of proton-bearing oxygenated functions was shown by the IR absorption bands at 3500 and 3280 cm^{-1} and the presence of two D_2O-exchangeable broad signals in the ^1H NMR spectrum (δ 6.76 and 5.10) (Table 1). Three one-proton signals were observed in the region of protons geminal to oxygen (δ 4.73, *br s*; 4.43, *t*, $J = 9.0$ Hz; 4.23, *d*, $J = 9.0$ Hz) and three corresponding doublets in the region of the sp^3-hybridized carbons carrying oxygen atoms (δ 84.8, 82.3, 72.4). Examination of the reported data suggested that **1** was a hydroperoxy-hydroxy-γ-lactone. In agreement with this structure the diacetate **2**, obtained upon acetylation of **1** by acetic anhydride–4 dimethylaminopyridine showed in its IR spectrum three distinctive and characteristic carbonyl bands: 1790 (Me–CO–OOR) [4], 1765 (γ-lactone) and 1740 (Me–CO–OR) cm^{-1}.





3

	R	R^1
1	–OH	–H
2	–OAc	–Ac
4	–OMe	–H

The ^{13}C NMR spectrum of **1** showed the presence of 15 carbon atoms (Table 2). In addition to the ones already referred to above the others were: one methyl, four methylenes, one methine, one aliphatic quaternary carbon and two olefinic carbons.

Acetylation of **1** by acetic anhydride–pyridine afforded the unstable monoacetyl derivative **3** (IR bands at 1740 and 1250 cm^{-1}, three-protons singlet at δ 2.00), whose UV and IR spectra [UV λ_{max}^{EtOH} nm (log ε): 238 (3.6), 310 (30): IR absorption band at 1695 cm^{-1}] suggested the presence of an α,β-unsaturated ketone, arising from an allylic hydroperoxide by elimination of acetate under these acyl-

Table 1. ^{1}H NMR spectral data for compounds **1–4** (200 MHz, $CDCl_3$ except **1** in C_5D_5N, TMS as int. standard)

	1	2	3	4
H-1	4.73*brs*	4.69*brt*	—	4.49*dd*
H-2a	*	1.60*m*	2.04*m*	1.62*m*
H-2b	*	*	*	*
H-3a	1.32*dt*	1.30*m*	*	*
H-3b	*	*	*	*
H-5	4.23*d*	5.15*d*	5.14*d*	3.91*dd*
H-6	4.43*t*	4.17*dd*	4.22*dd*	4.19*dd*
H-7	2.62*m*	2.87*tq*	2.95*tq*	2.65*tq*
H-8a	*	2.50*dq*	*	2.40*dq*
H-8b	*	*	*	*
H-9	5.76*t*	5.78*t*	6.18*t*	5.68*t*
H-13a	6.26*d*	6.20*d*	6.20*d*	6.22*d*
H-13b	5.45*d*	5.48*d*	5.46*d*	5.53*d*
H-14a	2.50*d*	2.32*d*	2.13*s*	2.21*d*
H-14b	2.38*d*	2.26*d*		2.16*d*
H-15	1.18*s*	1.07*s*	1.18*s*	0.98*s*
OOH	6.76*br*	—	—	—
OH	5.10*br*	—	—	2.27*d*
OOAc	—	2.10†	—	—
OAc	—	2.02†	2.00	—
OOMe	—	—	—	3.80

*Could not be assigned because of overlapping.

†Assignments are interchangeable.

Most of the coupling constants were virtually identical for compounds **1–4**; those given for **1** are taken as being representative. Values for those signals that changed significantly in **2–4** or those signals that were obscured in **1**, are also given: *J* (Hz) for **1**: 2a,3a = 14; 2b,3a = 5; 3a,3b = 14; 5,6 = 9.0; 6,7 = 9.0; 7,13a = 3.3; 7,13b = 3.1; 8a,9 = 7.5; 8b,9 = 7.5; 14a,14b = 13; compound **2**: 5,6 = 8.0; 7,8a = 2; 8a,8b = 13; 8a,9 = 7.8; 8b,9 = 7.8; compound **3**: 5,6 = 8.2; compound **4**: 5,OH = 3.0; 5,6 = 8.1.

Table 2. ^{13}C NMR spectral for compounds **1** and **2** (50.3 MHz, C_5D_5N for **1**, $CDCl_3$ for **2**, TMS as int. standard)

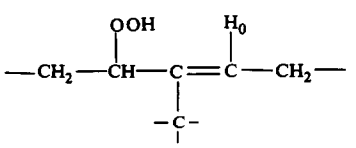
	1	2
C-1	84.8*d*	84.6*d*
C-2	29.0*t*	27.7*t*
C-3	31.5*t*	30.3*t*
C-4	41.5*s*	39.6*s*
C-5	72.4*d*	72.0*d*
C-6	82.3*d*	79.0*d*
C-7	51.1*d*	49.6*d*
C-8	24.8*t*	24.8*t*
C-9	121.3*d*	122.6*d*
C-10	140.2*s*‡	138.5*s**
C-11	142.1*s*‡	139.2*s**
C-12	169.5*s*	168.4*s*
C-13	118.3*t*	119.5*t*
C-14	33.2*t*	32.5*t*
C-15	23.1*q*	22.5*q*
OAc	—	167.8*s*†
		20.4*q*§
OOAc	—	171.2*s*†
		17.7*q*§

*,†,‡,§Assignments with the same sign are interchangeable.

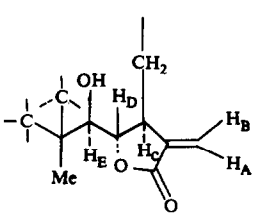
ating conditions [5]. The ^{1}H NMR spectrum of **3** showed, by comparison with the spectrum of **1**, loss of the broad one-proton singlet at δ 4.73, which was therefore assigned to the proton on the carbon bearing the hydroperoxy function. Significant paramagnetic shifts of the triplet at δ 5.76 ($\Delta\delta$ = 0.42 ppm) and the doublet at 4.23 ($\Delta\delta$ = 0.91 ppm), permitted their assignments to the olefinic proton H_o on the partial formula **A** and to the proton geminal to the hydroxyl group, respectively. The coupling constant of the triplet (J = 7.5 Hz) showed that its multiplicity was due to vicinal coupling with two protons. Irradiation of this signal in **1** caused the broad singlet at 4.73 to collapse to a broad triplet, suggesting the presence of another methylene group, adjacent to the carbon bearing the hydroperoxy function.

The arrangement of the substituents about the α,β-unsaturated γ-lactone was clarified by the following double-resonance experiments, that led to the partial formula **B**.

Irradiation of the doublet at δ 6.26 (H_a) caused the multiplet at 2.62 to be simplified to a broad, but not well resolved, triplet; a similar simplification was obtained by irradiation of the doublet at δ 5.45 (H_b), so locating the H_c proton at 2.62. Saturation of this signal converted the H_a and H_b doublets into singlets, and changed the one proton triplet at δ 4.43 to a doublet (J = 9.0 Hz), assigning this signal to the lactone methine H_d. Saturation of the signal at δ 2.62 caused the zone between 2.0 and 2.3 ppm to change, but, due to the complexity of this part of the spectrum, no assignment could be made. However, the multiplicity of the H_c signal suggested the presence of an adjacent methylene group in addition to the carbon bearing H_d. Irradiation at δ 4.43 simplified the multiplet at δ 2.62 to a very broad doublet, and collapsed the doublet at δ 4.23 to a sharp singlet. Saturation of this signal afforded the expected collapse of the triplet at δ 4.43 to a doublet (J = 9.0 Hz); the doublet at δ 4.23 was therefore assigned to H_e, on the carbon bearing the hydroxyl group and adjacent to a quaternary carbon. The only methyl group in **1**



A



B

was observed as a sharp singlet at δ 1.18: both sharpness and position of this signal showed it was on an aliphatic quaternary carbon, and it was therefore placed on the carbon adjacent to the one bearing H_e, that was the only aliphatic quaternary carbon in **1**.

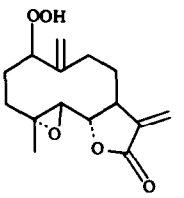
Irradiation of the triplet at δ 5.76 and of the multiplet at δ 2.62 caused similar changes in the methylene region between 2.0 and 2.3 ppm, suggesting the presence of a common allylic methylene between the olefinic methine in partial formula **A** and the aliphatic methine bearing H_c in partial formula **B**. This was clearly seen in the spectra of the diacetate **2** and the monomethyl derivative **4**, where one proton of this methylene was recognizable as a doublet of quartets, whose simplification to a doublet of doublets upon irradiation of the two methines in question allowed the unambiguous intercorrelation of the resonance signals of H_o and H_c.

Another methylene was observed as an isolated AB system (δ 2.50 and 2.38, $J = 13$ Hz), necessarily placed between the tertiary olefinic carbon in partial formula **A** and the quaternary aliphatic carbon in partial formula **B**. In keeping with this, irradiation of the proton geminal to the hydroperoxyl group afforded a sharpening of the AB doublets, consistent with the elimination of a long-range coupling, while irradiation of all the other signals present in the ^{1}H NMR spectrum did not have any influence on this spin system.

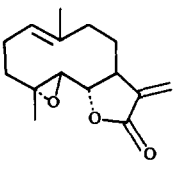
The last methylene had to be placed between the remaining free points of partial formulas **A** and **B**, thus leading to the final cyclogermacrane structure **1** for crispolide.

The stereochemistry at the chiral centres bearing hydrogen atoms was deduced from the corresponding coupling constants in the ^{1}H NMR spectrum. As the multiplicity of the H-1 signal was not well resolved in **1**, the β-configuration of the hydroperoxyl group in **1** was assigned by analogy with the β-configuration of this group in its derivatives **2** ($J_{1,2} = 3.6$ Hz) and **4** ($J_{1,2a} = 2.5$ Hz; $J_{1,2b} = 4.0$ Hz). It was assumed that no isomerization had taken place during acetylation or methylation of the hydroperoxy function. The stereochemistry at the quaternary carbon, C-4, was not unambiguously determinable by NMR data, although a syn relationship between the C-4 methyl and the C-5 hydroxyl seems to be likely, as comparison of the ^{1}H NMR spectra of **2** and **3** with the one of **4** show appreciable γ-acetylation shifts ($\Delta\delta = 0.09$ and 0.20 ppm) for this methyl group.

Compound **1** is rather insoluble in low and medium polarity solvents, and only moderately soluble in higher polarity solvents such as pyridine or DMSO, where a marked decomposition takes place in a few days. A thorough analysis of the ^{13}C NMR spectrum was therefore accomplished on the derivative **2**, which displayed a higher stability in deuterochloroform solution. Apart from the acetate and peracetate groups and the two tertiary olefinic carbons, all the other carbon resonances have been assigned for **2** by chemical shift and multiplicity considerations and by selective decouplings. The ^{13}C NMR spectral assignments for **1** were deduced from comparison of the chemical shifts and multiplicities of the signals in the two spectra. The upfield position (*ca* 5 ppm) of C-1 with regard to the corresponding carbons in a series of 1-hydroperoxy-$\Delta^{10(14)}$-germacranolides [6] is probably due to the endocyclic nature of the double bond in **1**, causing reduced interaction between the π-type n orbital of the oxygen and the vacant π^* orbital of the double bond, with consequent reduced deshielding of the C-1 carbon [6, 7].



5



6

Compound **1**, whose unusual modified germacrane skeleton has only recently been reported to occur in disyhamifolide [8], can be formed from peroxyparthenolide **5**, the product of the photo-oxygenation of parthenolide **6** [6], by an acid catalysed transannular cyclization involving the C-10 exocyclic methylene and the 4,5-epoxide group. Inspection of models shows the possibility of this cyclization occurring in the all-chair conformation of 4,5-epoxy-$\Delta^{10(14)}$-germacranolides. In this connection it is also noteworthy that **6** is the main sesquiterpene lactone isolated both from *T. vulgare* var. *crispum* and the two chemotypes of *T. vulgare* investigated. Since chloroform always contains traces of hydrochloric acid, it became questionable whether **1** was a genuine plant product or an artefact due to the treatment of **5** with large amounts of this solvent.

The genuine presence of **1** in plant material was ascertained by TLC analysis of alcohol extracts of the plant and spraying the plates with ferrous thiocianate. Only one peroxide-positive zone appeared, whose R_f corresponded to that of **1**, which is quite different from the R_f of **5** with the eluent used (8% ethanol in chloroform). On the other hand the simple treatment with chloroform failed to produce **1** from **5**, which was isolated from a plant extract using chromatographic techniques and eluents similar to the ones used in the isolation of **1** [6].

The production of peroxides seems to be an important taxonomic characteristic for *T. vulgare*. At present 96 samples of this plant have been investigated with regard to their contents in sesquiterpene lactones, and according to TLC and ^{1}H NMR analysis of the lactone extracts, at least nine different chemotypes have been noticed. Only 23 of them belong to the two peroxide-producing chemotypes from which **1** was isolated.

EXPERIMENTAL

Mps are uncorr. Si gel 60 (70–230 mesh) was used for CC. Si gel 60 pre-coated plates were used for prep. TLC (thickness: 2 mm); ^{1}H and ^{13}C NMR spectra were run at 200 and 50.3 MHz respectively.

Plant material. T. vulgare var. *crispum* was bought from Società dell'Alpestre (Carmagnola, Torino); *T. vulgare* chemotypes were collected at Carmagnola, Torino (chemotype A) and near Mt. Bracco, Torino (chemotype B) during

August 1979. Plant material was identified by Professor T. Sacco. Voucher specimens are kept at the Herbarium of the Istituto di Botanica Speciale Veterinaria, Università di Torino.

Isolation of crispolide (1). Dried non-woody aerial parts (leaves and flowers, 3 kg) were extracted with $CHCl_3$ (1 × 15 l., 3 × 10 l.) at room temp. The tarry residue remaining after removal of the solvent at red. pres. was purified by standard procedures [9], affording a thick syrup that was chromatographed on a Si gel (400 g) column, eluted with $CHCl_3$ containing increasing amounts of MeOH. Fractions eluted with $CHCl_3$–MeOH (9:1) yielded **1** (270 mg from *T. vulgare* var. *crispum*, 90 mg from chemotype A of *T. vulgare* and 80 mg from chemotype B).

In addition to **1**, **6** (4 g) and artemorin (60 mg) were obtained from *T. vulgare* var. *crispum*; **6** (5 g) and artemorin (80 mg) from chemotype A of *T. vulgare* and **6** (4 g), artemorin (80 mg) and tatridin A and B (2 g) from chemotype B.

Crispolide **1**. 1β - Hydroperoxy - 5β - hydroxy - 4,14 - cyclogermacra - 9,11 - dien - 6,12 - olide, mp: softened at 152°, but did not melt when heated up to 300°; $[\alpha]_D^{24} - 20°$ (pyridine; *c* 0.90); IR ν_{max}^{KBr} cm^{-1}: 3500, 3280, 1745, 1650, 1155, 965; UV λ_{max}^{EtOH} nm (log ε): 210 (4.06); EIMS 70 eV, *m/z* (rel. int.): no molecular ion, 264 $[M-O]^+$ (26), 263 $[M-OH]^+$ (6), 262 $[M-H_2O]^+$ (30), 247 $[M-H_2O-Me]^+$ (28).

Diacetylcrispolide **2**. To a magnetically stirred suspension of **1** (42 mg, 0.15 mM) in CH_2Cl_2 (3 ml) 4-dimethylaminopyridine (55 mg, 0.50 mM) and Ac_2O (110 μl, 0.100 mM) were added at room temp. After 10 min the sol. was diluted with $CHCl_3$ and poured into a 5% aq. soln of $NaHCO_3$. The organic phase was separated, washed with H_2O, dried over dry $MgSO_4$ and evaporated. The residue was purified by prep. TLC ($CHCl_3$–Me_2CO 6:1), affording 35 mg **2**; mp: did not melt when heated up to 300°; $[\alpha]_D^{24} - 27°$ ($CHCl_3$; *c* 0.60); IR ν_{max}^{KBr} cm^{-1}: no OH bands, 1790, 1765, 1740, 1650, 1250, 1240, 1180, 970; UV λ_{max}^{EtOH} nm (log ε): 210 (4.03); EIMS 70 eV, *m/z* (rel. int.): 364 $[M]^+$ (12), 304 $[M-60]^+$ (18), 262 $[M-60-42]^+$ (30), 244 $[M-60-60]^+$ (26).

Acetylanhydrocrispolide **3**. (a) From **1**. A 50 mg sample of **1** was dissolved in 1.5 ml pyridine, and 2 ml Ac_2O were added to the mixture. After standing overnight, ice was added to the reaction mixture, which was then extracted with $CHCl_3$ and washed with 10% aq. $NaHCO_3$, H_2O, diluted HCl, H_2O and then dried over dry $MgSO_4$ and evaporated, affording 48 mg of **3** as an an unstable colourless oil, rapidly resinifying.

(b) From **2**. A 20 mg sample of **2** was adsorbed on a prep. TLC plate and left there for 1 day; after elution of the plate ($CHCl_3$–Me_2CO, 6:1) **3** was obtained in an almost quantitative yield. $[\alpha]_D^{24} + 9°$ ($CHCl_3$; *c* 0.80); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: no OH bands, 1770, 1740, 1695, 1640, 1250, 1100, 950; UV λ_{max}^{EtOH} nm (log ε): 210 (4.02), 238 (3.6), 310 (ε = 30) EIMS: 70 eV, *m/z* (rel. int.): 304 $[M]^+$ (8), 276 $[M-28]^+$ (25), 244 $[M-60]^+$ (30).

Methylcrispolide **4**. Compound **1** (5 mg) was suspended in 0.5 ml $CHCl_3$ and the suspension stirred with 30 mg Ag_2O and 30 μl MeI for 24 hr at room temp. After filtering, the reaction mixture was evaporated affording 2 mg **4**: mp 157°, $[\alpha]_D^{24} + 6°$ ($CHCl_3$; *c* 0.35); IR ν_{max}^{KBr} cm^{-1}: 3700, 3400, 1770, 1650, 1130, 970; UV λ_{max}^{EtOH} nm (log ε): 212 (4.10); EIMS: 70 eV, *m/z* (rel. int.): 294 $[M]^+$ (8), 263 $[M-31]^+$ (15), 247 $[M-31-16]^+$ (36).

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REFERENCES

1. Nano, G. M., Bicchi, C., Frattini, C. and Gallino, M. (1976) *Planta Med.* **30**, 211.
2. Nano, G. M., Bicchi, C., Frattini, C. and Gallino, M. (1979) *Planta Med.* **35**, 270.
3. Nano, G. M., Appendino, G., Bicchi, C. and Frattini, C. (1980) *Fitoterapia* **LI**, 135.
4. Davison, W. H. T. (1951) *J. Chem. Soc.* 2456.
5. Doskotch, R. W., El-Feraly, F. S., Fairchild, E. H. and Huang, C. T. (1977) *J. Org. Chem.* **42**, 3614.
6. El-Feraly, F. S., Chan, Y. M., Capiton, G. A., Doskotch, R. W. and Fairchild, E. H. (1979) *J. Org. Chem.* **44**, 3952.
7. Senda, Y., Isiyama, J. and Imaizumi, S. (1978) *Tetrahedron Letters* 1805.
8. Bohlmann, F., Dhar, A. K., Jakupovic, J., King, R. M. and Robinson, H. (1981) *Phytochemistry* **20**, 1077.
9. Mabry, T. J., Miller, H. E., Kugan, H. B. and Renold, W. (1966) *Tetrahedron* **22**, 1139.