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A NOVEL ENOL-PSEUDOGUAIANOLIDE FROM *PSILOSTROPHE COOPERI*

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Key Word Index—*Psilostrophe cooperi*; Asteraceae; sesquiterpene lactones; helenanolides; mutagenic activity.

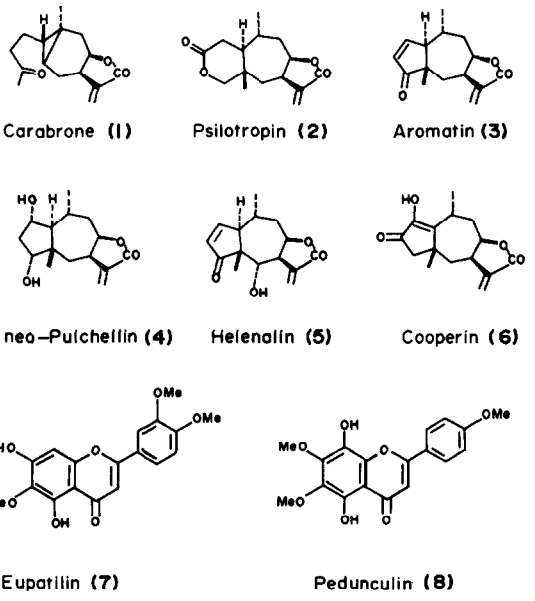
Abstract—Chemical studies of *Psilostrophe cooperi* afforded in addition to psilotropin, four unreported helenanolides, two methylated flavonoids and a new pseudoguaianolide, cooperin, with an unusual enol-moiety. The structure of cooperin was established by spectroscopic methods and confirmed by X-ray analysis. In addition, all the sesquiterpene lactones were tested for mutagenic activity.

INTRODUCTION

Psilostrophe (Asteraceae) is a small genus distributed throughout the southwest United States and the northern part of Mexico [1, 2]. Three species, *P. cooperi* (Gray) Green, *P. villosa* Rydb. and *P. gnaphaloides* DC. have been investigated recently [3–5] and have been found to be closely related to the genus *Hymenoxys*. Reinvestigation of *Psilostrophe cooperi*, from central Baja California, Mexico, resulted in isolation of the major constituent psilotropin **1**, previously reported from *P. cooperi* and *P. gnaphaloides*, and five unreported pseudoguaianolides, and cooperin **6**, a novel enol-pseudoguaianolide.

RESULTS AND DISCUSSION

Chloroform extraction and column chromatography of *Psilostrophe cooperi* yielded psilotropin **1**, carabrone **2**, aromatin **3**, neopulchelin **4**, helenalin **5**, and two flavonoids eupatilin **7** and pedunculin **8** [(6–11)]. In addition a new pseudoguaianolide, which we named cooperin **6** was isolated. Cooperin **6**, mp 202°, gave a molecular ion at *m/z* 262 with a molecular formula of $C_{15}H_{18}O_4$. The presence of an α-methylene-γ-lactone moiety was indicated by IR (1755 and 1650 cm^{-1}), ^1H NMR (one proton doublets at 6.29 and 5.56; $J_{13a/7} = 3$ H and $J_{13b/7} = 2.7$ Hz) and ^{13}C NMR (carbonyl signal at 169 ppm). An additional carbonyl group was shown by IR (1700 cm^{-1}) and ^{13}C NMR (singlet at 201.54 ppm). The ^1H NMR spectral data (Table 1) of the protons H-6 to H-13 were almost identical with those of psilotropin **1** and compounds **2–5**. Therefore, a pseudoguaianolide structure with a *cis*-fused 7,8-α-methylene-γ-lactone ring, closed to the C-8 would be accordance with same K-s rule [12]. Assignment of the H-7 (δ2.89–2.98) was achieved by spin decoupling at the frequency of the vinylic protons (Table 1). Irradiation at δ2.95 collapsed the vinyl proton doublets and a three proton doublet at δ1.30 which were assigned to the methyl group in position C-10. The signals of H-7 were partially overlapped



by those of H-10. This was confirmed by the integration which showed two protons for the irradiated multiplet. Furthermore, the complex signal at δ4.63 was changed to a double doublet (*dd*) and an ABX system at δ1.92 simplified to an AB system. These signals were assigned to H-8, which was shifted downfield because of the lactone ring closing to C-8, and to H-6a and H-6b, respectively.

Irradiation at the frequency of the H-8 signal resulted in the change of one proton multiplet at δ2.3 to a double doublet which could be assigned to H-9b. Due to the coupling constant ($J_{9b/8} = 11.8$ Hz) a β-configuration of this proton was established. The H-9a was observed as a three proton multiplet at δ1.85–2.0 partially overlapped by H-6a and H-6b. An AB system for a methylene group at 2.37 without any coupling with other signals required the presence of two neighbouring quarternary C-atoms, at least one of which had to be, considering the chemical

Dedicated to the memory of the late Professor Tony Swain, a great scholar, mentor and friend.

Table 1. ^{1}H NMR spectra data of cooperi **6** (300 MHz, $CHCl_3$, TMS as internal standard)

H	
4a	2.34 *d*
4b	2.40 *d*
6a	1.88 *dd*
6b	1.98 *dd*
7, H-10	2.88–2.98 *m*
8	4.64 *t*
9a	1.91 *t*
9b	2.21 *t*
13a	5.56 *d*
13b	6.29 *d*
14	1.54 *d*
15	1.30 *s*
OH	5.61 *br*

Coupling constants, *J* (Hz): 4a, 4b = 18.9; 6a, 6b = 14.7; 6a, 7 = 3.9; 6b, 7 = 12; 7,8 = 8.1; 8,9b = 11.8; 8.9a = 3.6 10,14 = 7.2; 13a,7 = 3.0; 136,7 = 3.3.

Table 2. ^{13}C NMR data for cooperin **6** (300 MHz, $CHCl_3$, TMS as internal standard)

C		C	
1	150.15 *s*	9	40.68 *t*
2	148.22 *s*	10	27.80 *d*
3	201.69 *s*	11	138.74 *s*
4	46.70 *t*	12	169.53 *s*
5	40.93	13	122.83 *t*
6	35.41 *t*	14	19.59 *q*
7	38.22 *d*	15	28.69 *q*
8	76.43 *d*		

*Assignments may be interchanged.

shift, a carbonyl of a double bond carbon. The position of the relatively downfield shifted signals for H-10 and H-14 could be explained by the presence of a deshielding double bond in position C_1 and C_2. With the exception of a singlet at δ1.30 assignable to the methyl group in position C-5, the only signal left was one proton singlet at δ6.2 which in the H NMR spectrum occasionally appeared. The use of D_2O exchange to identify the OH-group was not successful. However, characteristic absorptions in the IR (3500 and 1650 cm^{-1}) [13] and UV spectra (λ_{max} = 210 and 260 nm) and a positive colour reaction performed with ethanolic ferric chloride solution [14] showed the presence of an enol group, which, according to the ^{1}H NMR data, could be assigned to position C-2. This was confirmed by a ^{13}C NMR-DEPT analysis which in the range of 120–205 ppm revealed five quaternary C-atoms. Two of them were identified as carbonyl groups (201.54 and 169.44 ppm) and one could be assigned to C-12 (138.8 ppm). The other two quarternary carbons (148.17 and 149. 96 ppm), required the presence of a completely substituted double bond in position 1,2. On the basis of these data, structure **6** could be proposed,

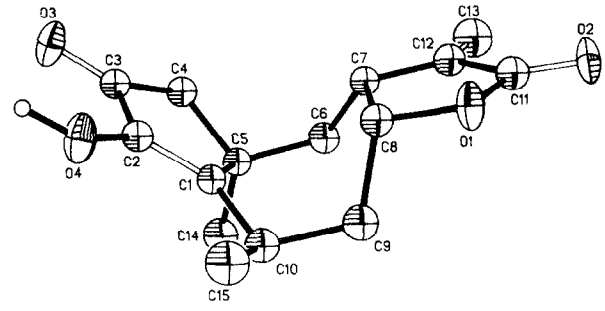


Fig. 1. Stereoscopic view of cooperin **6**.

nonetheless it was still uncertain if the carbonyl group was in position 3 and the methylene group (2.37) in position 4 or if the opposite were true. The chemical shift at δ2.37 and the results of a 500 MHz ^{1}H NMR spectrum, where irradiation of one proton of the isolated methylene group in position C-4. To confirm the NMR assignments and to get more information of configurational and conformational conditions, an X-ray analysis of cooperin **6** was undertaken.

Figure 1 is a stereoscopic drawing of cooperin **6** which definitely shows that the methylene group (^{7}H NMR: 2.35 ppm) is located in position 4 and the carbonyl group in position 3 of the planer cyclopentenone ring. The enol group in position 2, assumed to show up only is solution, is also present in the crystal. The α-methylene-γ-lactone moiety is 7,8-*cis* fused to the seven membered main ring which adopts boat conformation. The bond distance and angles (Tables 3 and 4) in the cyclopheptane and lactone ring are not unusual and show good agreement with corresponding fragments in similar compounds [15, 16]. The orientation of the substituents at C-5, C-7 C-8 and C-10 is the same as proposed in formula **6** and corresponds with the relative stereochemistry of the compounds **1**–**5**. The packing of the molecules in the unit cell is shown in Fig. 2. The hydroxyl hydrogen in position 0-4 appears to participate in hydrogen bonding to the oxygen 2 of a neighbouring molecule (Table 3; H-04 . . . 0–2 = 1.98 Å) [17]. The absolute configuration of cooperin was not assignable from the results of the X-ray investigation.

Mutagenic activity

Five of the isolated sesquiterpene lactones, psilotropin **1**, carabrone **2**, aromatin **3**, helenalin **5**, and cooperin **6**

Table 3. Interatomic distances (Å) with Esd's for cooperin **6**

C(01)–C(02)	1.364(8)	C(07)–C(12)	1.495(9)
C(01)–C(10)	1.524(9)	C(07)–C(08)	1.555(9)
C(01)–C(05)	1.526(9)	C(08)–O(01)	1.465(8)
C(02)–O(04)	1.364(7)	C(08)–C(09)	1.588(10)
C(02)–C(03)	1.470(9)	C(09)–C(10)	1.552(10)
C(03)–O(03)	1.233(8)	C(10)–C(15)	1.536(11)
C(03)–C(04)	1.493(9)	C(11)–O(02)	1.195(8)
C(04)–C(05)	1.577(9)	C(11)–O(01)	1.357(9)
C(05)–C(06)	1.539(9)	C(11)–C(12)	1.491(10)
C(05)–C(14)	1.559(10)	C(12)–C(13)	1.320 (11)
C(06)–C(07)	1.543(9)	O(02) . . . H(40)	1.977

Table 4. Interatomic angles with Esd's for cooperin **6**

C(02)–C(01)–C(10)	129.5(6)	C(12)–C(07)–C(06)	112.9(6)
C(02)–C(01)–C(05)	109.6(5)	C(12)–C(07)–C(08)	103.1(5)
C(10)–C(01)–C(05)	120.7(6)	C(06)–C(07)–C(08)	113.2(5)
C(01)–C(02)–0(04)	129.0(6)	0(01)–C(08)–C(07)	105.9(5)
C(01)–C(02)–C(03)	112.7(6)	0(01)–C(08)–C(09)	106.4(6)
0(04)–C(02)–C(03)	118.2(5)	C(07)–C(08)–C(09)	116.1(5)
0(03)–C(03)–C(02)	124.9(6)	C(10)–C(09)–C(08)	112.2(6)
0(03)–C(03)–C(04)	128.4(6)	C(01)–C(10)–C(15)	114.7(6)
C(02)–C(03)–C(04)	106.7(5)	C(01)–C(10)–C(09)	111.4(6)
C(03)–C(04)–C(05)	105.7(5)	C(15)–C(10)–C(09)	108.8(6)
C(01)–C(05)–C(06)	114.3(5)	0(02)–C(11)–0(01)	121.7(7)
C(01)–C(05)–C(14)	108.7(5)	0(02)–C(11)–C(12)	130.5(7)
C(01)–C(05)–C(04)	103.4(5)	0(01)–C(11)–C(12)	107.8(6)
C(06)–C(05)–C(14)	109.3(6)	C(13)–C(12)–C(11)	120.0(7)
C(06)–C(05)–C(04)	109.7(5)	C(13)–C(12)–C(07)	130.3(7)
C(14)–C(05)–C(04)	111.4(5)	C(11)–C(12)–C(07)	109.6(6)
C(05)–C(06)–C(07)	116.5(5)	C(11)–0(01)–C(08)	113.1(5)

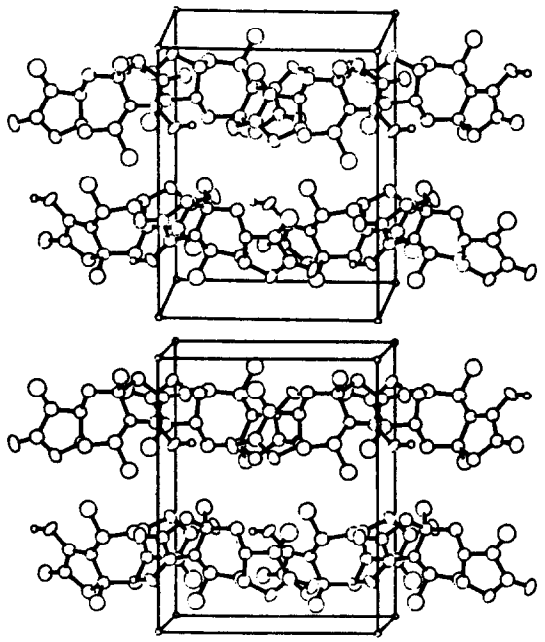


Fig. 2. Molecular packing of cooperin **6** in the unit cell.

were tested for mutagenic activity on *Salmonella typhimurium* strains (TA 98, TA 100, TA 102 and TA 104) as described by Maron and Ames [18]. With the exception of carabrone **2**, all compounds showed no activity in the spot test. Carabrone **2**, gave a response with strain TA 104, confirmed as a dose response relationship in a plate incorporation test (Table 5). Tests with and without S9 mix containing 4% rat liver S9 yielded the same results, showing that no metabolic activation is necessary for the mutagenic effect. Application of 10 μg of the positive control methylglyoxal [19] per plate resulted in 531 coloniesThe same amount of colonies found with 100 μg of carabrone **2** per plate shows that the activity of carabrone is the only compound with an open ring system

Table 5. Mutagenicity of carabrone **2** on strain TA 104

μg compound per plate	Revertants per plate Without S9 mix	With S9 mix
—	326 (16.2)	293 (13.3)
25	359 (18.7)	373 (20.6)
50	422 (18.7)	417 (18.3)
75	459 (19)	479 (19.8)
100	530 (20.5)	532 (18.6)

containing an acetyl group. It was not surprising to find mutagenic activity in the base substitution strain TA 104, since this strain has been reported to detect naturally occurring carbonyl mutagens efficiently [19].

EXPERIMENTAL

Psilostrophe cooperi was collected in March, 1986, one mile northeast of Catavina, Baja California Norte, Mexico, near Highway 1 and identified by Dr Jan West (UC Irvine). Voucher specimen no. 22991 is deposited at the Museum of Systematic Biology, UC Irvine. Air-dried leaves, stems and flowering heads (800 g) were extracted with $CHCl_3$ and worked-up as previously reported [20], providing 12.5 g of crude gum. The crude extract was dissolved is a small amount of $CHCl_3$–Me_2CO (9:1) and chromatographed on a silica gel column. 20 ml fractions were collected as follows: 1–300 $CHCl_3$–Me_2CO (9:1) and 300–450 $CHCl_3$–Me_2CO (4:1). Fractions 70–85 afforded 20 mg of carabrone **2** and fractions 166–265 gave 2 g of psilotropin **1**, which was recrystallized from EtOAc. Fractions 104–121 and 356–450 were rechromatographed over Sephadex LH 20 (MeOH) and yielded 20 mg of aromatin **3** and 60 mg of neopulchelin **4**, respectively. The same chromatographic conditions were used to isolate 50 mg of helenalin **5** from fractions 266–355, which contained in addition the two flavonoids eupatilin **7** and pedunculin **8** and the sesquiterpene lactone cooperin **6**. Cooperin **6** was purified over a silica gel column using hexane–EtOAc (1:1) as solvent. *Ca* 20 mg of pure compound were obtained and crystallized from EtOAc–$CHCl_3$. The two flavonoids were separated by Sephadex column chromatography (MeOH) followed by a silica gel chromatography with hexane and EtOAc. Compounds **1**–**5** were identified by comparing the ^{1}H NMR spectra with those reported in the literature [3–9]. The two flavonoids were identified by their UV and ^{1}H NMR spectra and compared to reference data [10, 11]. Cooperin **6**: Colourless crystals ($CHCl_3$–EtOAc), mp 202° UV λ nm: 210, 260; IR cm^{-1}: 3480, 1755, 1650; CIMS *m/z* (rel. int.) 263 (M + 1) (100); EIMS *m/z* (rel. int.): 262 (31); 243 (14); 191 (8); 189 (9); 164 (7); 162 (8); 151 (10); 145 (12); 137 (10); 123 (7); 119 (11); 109 (25); 107 (10); 105 (12); 95 (11); 93(16); 91 (20); 81 (36); 79 (21); 77 (16); 69 (12); 67 (20); 65 (8).

X-Ray analysis

A colourless crystal of approximate dimensions 0.27 × 0.37 × 0.60 mm was cleaved from a larger crystal and mounted on a glass fibre. It was accurately aligned on the Nicolet P3 automated four-circle diffractometer at the University of California, Irvine. Subsequent setup operations (determination of acurate unit cell dimensions and orientation matrix) and collection of room temperature (21°) intensity data were carried out using standard techniques similar to those of ref. [22]. Final cell parameters are based on a least-squares analysis of 25 reflections in well-separated regions of reciprocal space, all having 24° < 20° < 28°. Details are given in Table 6.

Table 6. Experimental data for the X-ray diffraction of cooperin **6**

Formula: $C_{15}H_{18}O_4$
M_r: 262.33
Crystal system: orthorhombic
Space group: $P2_12_1$ (No. 19; D_2^4)
$a = 9.7356(12)$ Å
$b = 10.5704(14)$ Å
$c = 13.3900(24)$ Å
$V = 1327.0(3)$ Å^3
$Z = 4$
D_{calcd}, g/cm^3 = 1.31
Diffractometer: Nicolet P3
Data collected: $+h$, $+k$, $+l$
Scan type: coupled 0 (crystal)-2θ (counter)
Scan width: $[2\theta(K_1)-1.2]$- $[2\theta(K,+1.2]$
Scan speed: 4.0 deg/min (in 2θ)
$2\theta_{max}$, deg: 45.0
u (MoKα), cm^{-1} = 0.88
Unique reflections: 1027
Reflections with $I > 0$: 992
No. of variables: 97
$RF = 7.7\%$
$R_{wf} = 9.6\%$
Goodness of fit: 2.68

A careful survey of a preliminary data set revealed the systematic extinctions $h00$ for $h = 2n+1$, $o k o$ for $k = 2n+1$ and $00l$ for $l = 2n+1$. The space group is thus defined as $P2_12_1$ (D_2^4; No. 19). All 1027 unique reflections were converted to unscaled $|F_0|$ values following correction for Lorentz and polarization effects. A Wilson plot was used to place the data on an approximate absolute scale. Those 992 reflections with $I > 0$ were considered observed.

The structure was solved by direct methods using the the SHELXTL PLUS program set [23]. A single 'E-map' revealed the positions of all non-hydrogen atoms. Subsequent crystallographic calculations were performed using the locally modified version of the UCLA Crystallographic Computing Package [24] at the University of California, Irvine. The structure was refined using full-matrix least-squares methods. The weighing scheme using $p = 0.05$ has been previously described [25]. Hydrogen atoms were placed in calculated positions with d(C-H) = 0.95 Å (26). The hydrogen atom on O (04) was located from a difference-Fourier synthesis but was not refined. The model converged with $R_F = 7.7\%$ $R_{WF} = 9.6\%$ and $GOF = 2.68$ for 97 variables refined against those 992 data with $I > 0$. A final difference-Fourier synthesis was featureless.

The analytical scattering factors of Cromer and Waber [27] for neutral atoms were used throughout the analysis; both the real ($\Delta f'$) and imaginary ($i\Delta f''$) components of anomalous dispersion [27] were included for all non-hydrogen atoms.

Mutagenicity test

The mutagenicity methods used were essentially those of ref [18] with minor modifications. The *Salmonella typhimurium* strains TA 98, TA 100, TA 102 and TA 104 were obtained from Dr Bruce Ames (University of California, Berkeley). Rat liver S9 was kindly provided by Dr Ronald E. Rassmussen (University of California, Irvine). Spot tests were performed by dissolving the coupounds in dimethylsulphoxide (DMSO) (2 mg/ml) and pipetting 5 μl onto a sterile 0.5 cm filter paper disk, which was placed in the center of the bacterial test plate. Positive control compounds in the spot test were: 2-aminofluorene (2-AF) and methyl methanesulphonate (MMS) and in the incorporation test: methylglyoxal (19).

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