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A NEW SCALARANE SESTERTERPENOID FROM THE MARINE SPONGE *CACOSPONGIA MOLLIOR*

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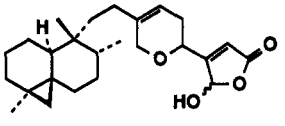
ABSTRACT.—A new sesterterpene, 12-deacetoxyscalaradial [**2a**] has been isolated from the sponge *Cacospongia mollior*, together with the previously described scalaradial [**2b**] and furoscalarol [**3**]. The structure of the new compound, proposed by spectral data, was confirmed by X-ray diffraction analysis. Compound **2a** is the first scalarane sesterterpenoid, without a substituent at C-12, to have been identified in nature. It shows high antifeedant activity and is hot to taste for the human tongue. Both **2a** and **2b** display cytotoxic activity.

In the course of our search for marine natural compounds that have biological activities, we have previously isolated and characterized a sesterterpenoid, cacospongionolide [**1**] (1,2), with high cytotoxic activity, from the sponge *Cacospongia mollior* Ridley (Dictyoceratida), collected in the northern Adriatic. We subsequently made an extensive collection of Dictyoceratida sponges in the Mediterranean Sea to find compounds related to cacospongionolide.

From a specimen of *C. mollior* collected near Naples, we have now isolated the new compound 12-deacetoxyscalaradial [**2a**], which shows interesting biological activities, together with two known scalarane sesterterpenoids, scalaradial [**2b**] and furoscalarol [**3**] (3). Here we describe the isolation and structure elucidation, including X-ray analysis, of the new compound [**2a**] and some of its biological activities.

RESULTS AND DISCUSSION

The Et_2O-soluble fraction of the Me_2CO extract of *C. mollior* was chromatographed on Si gel to give three scalarane sesterterpenoids, that in order of polarity were: 12-deacetoxyscalaradial (**2a**, 0.011% dry wt), furoscalarol (**3**, 0.21% dry wt) and scalaradial (**2b**, 0.98% dry wt). A preliminary analysis of nmr spectra showed a close similarity among the three compounds and strongly supported the presence of a scalarane skeleton for each of them. The structures of **2b** and **3** were confirmed by comparing their spectral data with published values (3,4).



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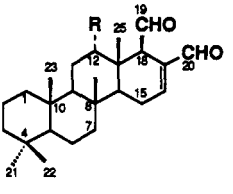
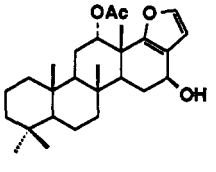


2a R=H
2b R=$OCOCH_3$



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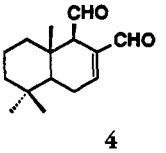

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12-Deacetoxyscalaradial [**2a**] was obtained as a solid, mp 200–203° (EtOH). Its molecular formula was deduced as $C_{25}H_{38}O_2$ from its mass spectrum (M^+, *m/z* 370) and its nmr data. Its ultraviolet absorption at 231 nm (ε 14000) and infrared bands at 1680 and 1640 cm^{-1} were characteristic of an enal moiety; further bands at 2845 and 1725 cm^{-1} in the infrared spectrum showed the presence of an aldehyde group. This was further substantiated by its ^{1}H-nmr spectrum that showed a doublet (δ 9.53) and a singlet (δ 9.45), long-range coupled with an olefinic proton (δ 7.10), and by its ^{13}C-nmr spectrum, with two carbonyl carbons as doublets (δ 202.0 and 193.1) and two olefinic carbons, a doublet (δ 154.28) and a singlet (δ 138.12).

The ^{1}H-nmr and ^{13}C-nmr spectra of **2a** (Table 1) were similar to those of **2b**, except for the absence of signals assigned to the acetoxy group. These data suggested that **2a** was the 12-deacetoxy derivative of scalaradial, the first such derivative identified in nature without a substituent at C-12. The ^{1}H-nmr spectrum of **2a** showed a complex AB system at δ 2.42 (Heq-15) and δ 2.32 (Hax-15), that, by a COSY-45 spectrum, was correlated with the olefinic proton (δ 7.10), the proton at C-14 (δ 1.22, dd) and was long-range coupled with the proton at C-18 (δ 2.80). The $J_{15ax\text{-}16}$ (2.4 Hz), $J_{15eq\text{-}16}$ (5.2 Hz), $J_{15ax\text{-}18}$ (3.8 Hz) and $J_{15eq\text{-}18}$ (2.0 Hz) coupling constants were determined by double-irradiation experiments.

The relative stereochemistry of the aldehyde group at C-18 was deduced from a

TABLE 1. Nmr Spectral Data of **2a** in $CDCl_3$ Solution.[a]

C	^{13}C	^{1}H	^{1}H Long-range correlated ($J_{C\text{-}H}$=10 Hz)[b]
1	39.80 t	1.69 m, 0.80 m	1.38 (H-2), 0.84 (H-23)
2	18.53 t	1.56 m, 1.38 m	0.80 (H-1)
3	42.06 t	1.34 m, 1.13 dt (13.5, 4.2)	1.69 (H-1), 0.80 (Hs-1, 22), 0.84 (H-21)
4	33.27 s	—	1.40–1.35 (Hs-2, 3, 6)
5	56.36 d	0.79 m	—
6	18.05 t	1.51 m, 1.35 m	0.94 (H-7)
7	41.70 t	1.74 dt (12.6, 3.3), 0.94 m	1.51 (H-6), 0.91 (H-24)
8	36.89 s	—	1.56 (H-11), 0.91 (H-24)
9	60.92 d	0.80	—
10	37.45 s	—	1.51 (H-6), 0.84 (H-23)
11	17.00 t	1.56 m, 1.34 m	—
12	41.15 t	1.90 dt (13.3, 3.2), 1.50 m	0.95 (H-25)
13	37.87 s	—	1.56 (H-11), 0.95 (H-25)
14	54.22 d	1.22 dd (11.7, 5.0)	1.90 (H-12), 1.50 (H-12), 0.95 (H-25)
15	24.29 t	ν_A 2.42 (20.4, 5.2, 5.0, 2.0)[c] ν_B 2.32 (20.4, 11.7, 2.4, 3.8)	—
16	154.28 d	7.10 m	2.42–2.32 (H-15)
17	138.12 s	—	9.45 (H-20), 2.80 (H-18), 2.42–2.32 (H-15)
18	60.78 d	2.80 br s	1.90 (H-12)
19	201.98 d	9.53 d (3.5)	2.80 (H-18)
20	193.07 d	9.45 s	7.10 (H-16)
21	33.25 q	0.84 s	—
22	21.32 q	0.80 s	1.34 (H-3)
23	16.42 q	0.84 s	—
24	16.02 q	0.91 s	—
25	16.99 q	0.95 s	—

[a]Chemical shifts are referred to TMS. Multiplicities are indicated by usual symbols. Coupling constants (Hz) are in parentheses.

[b]Only correlations not observed in the HETCOR nmr spectrum are reported.

[c]AB part of ABXYZ system.

NOESY nmr spectrum, that exhibited the presence of nOes (Table 2) indicating that the protons at C-18 and C-14 are oriented on the same side (α) of the molecule, while the aldehydic group at C-18 has the same orientation (β) as Me-13. From the NOESY spectrum it was possible to establish the unique assignment of chemical shifts of the two methyls at δ 0.91 and δ 0.95 from the presence of nOes between the aldehydic proton at δ 9.53 and the methyl at δ 0.95. Other nOes reported in Table 2 are in accord with the proposed structure, **2a**. Chemical shifts were assigned by COSY, NOESY and HETCOR nmr spectra, as reported in Table 1.

As the stereochemical assignments of some scalarane-type molecules, such as heteronemin (5) and foliaspongin (6), have been recently criticized on the basis of crystallographic studies of related compounds (5,7), we have also carried out the single

TABLE 2. Magnetization Exchange by Cross Relaxation (nOe) for **2a** (in $CDCl_3$ as observed from NOESY).[a]

Cross-peak coordinates below the diagonal δ_x–δ_y	Protons correlated
9.53–1.90	H-19, Hβ-12
9.53–0.95	H-19, Me-13
2.80–1.22	H-18, H-14
2.80–1.50	H-18, Hα-12
2.42–1.74	Hα-15, Hβ-7
2.32–0.95	Hβ-15, Me-13
2.32–0.91	Hβ-15, Me-8
1.74–0.91	Hβ-7, Me-8

[a] ^{1}H NOESY spectrum was recorded at 500 MHz. Only the cross-peaks not sensitive to strong filtering are reported.

crystal X-ray analysis of **2a**. A view of the final X-ray model of **2a** is presented in Figure 1 together with the atomic numbering scheme. The absolute stereochemistry of this molecule was not determined and the configuration shown is in accordance with that of other scalarane derivatives (8,9). The molecule exhibits a trans-fused tetracyclic sesterterpene skeleton, which has, in addition to the two methyl groups at C-4, three β-oriented angular methyl groups at C-8, C-10 and C-13, and two vicinal aldehydic substitutions, at positions 17 and 18 of the unsaturated ring *D*.

The chiral center C-18, which bears an equatorial aldehyde group, has the relative

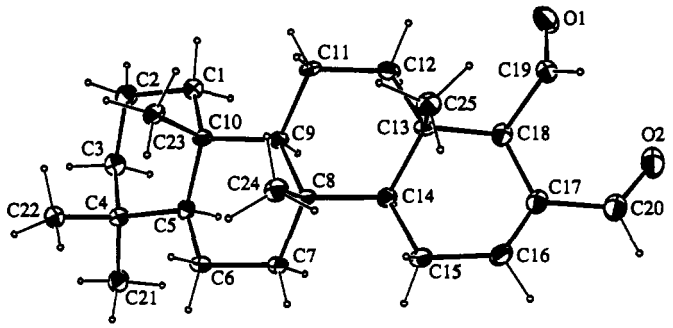

FIGURE 1. Perspective view of the final X-ray model of 12-deacetoxy scalaradial molecule [**2a**] with the atomic numbering scheme for non H-atoms. Thermal ellipsoids at the 30% probability are also shown.

configuration, *R**. The β-orientation of this group has been shown to be strictly correlated to the biological activity of this class of compounds (10).

The final position and equivalent isotropic thermal parameters for non H-atoms are reported in Table 3 and the molecular geometry is given in Table 4. Despite the relatively large estimated standard deviations on the positional parameters, probably caused by the accentuated mosaicity of the crystals, the general trend of the geometrical parameters agrees with the values reported for similar compounds. The presence in the tetracyclic system of four axial methyl groups (β-oriented), gives rise to skeletal distortions similar to those observed in other sesterterpenes with analogous sequences of axial substituents (9,11). The shortest methyl-methyl interactions are C-22·····C-23=3.22(1) Å, C-23·····C-24=3.17(1) Å, C-24·····C-25=3.22(1) Å; the most notable effects of the atomic crowding are the bond lengthening (see values in Table 4) in the region of the steric strain and the widening of the bond angles C-4–C-5–C-10=116.8(5)°, C-8–C-9–C-10=118.3(5)° and C-8–C-14–C-13=117.0(4)°.

Ring *A* adopts an almost ideal chair conformation, while in the other saturated rings, distortions from an ideal form include flattening of C-8 with respect to C-5 in ring *B* and of C-13 with respect to C-9 in ring *C*. The cyclohexene ring is in a slightly distorted half chair form, with C-13 and C-14 displaced 0.517(7) Å and 0.290(6) Å, respectively, in the opposite directions with respect to the best plane through the remaining ring atoms. The aldehydic C-O bond at C-17 is trans to the conjugated intracyclic double bond [O-

TABLE 3. Positional Parameters and Equivalent Isotropic Temperature Factors (Å^2) (e.s.d.s in parentheses for non-H atoms) of **2a**.

Proton	$B_{eq}=\frac{1}{3}\Sigma_i\Sigma_j B_{ij}a_i^*\mathbf{a}_i a_j^*\mathbf{a}_j$			
	x	*y*	*z*	B_{eq}
O-1[a]	0.500	0.100	0.200	9.2 (2)
O-2	0.934 (1)	0.467 (1)	0.0855 (5)	8.6 (2)
C-1	0.340 (1)	0.391 (1)	0.7523 (6)	6.1 (2)
C-2	0.318 (2)	0.419 (1)	0.8734 (6)	7.4 (2)
C-3	0.513 (1)	0.611 (1)	0.9289 (7)	6.9 (2)
C-4	0.542 (1)	0.804 (1)	0.8858 (6)	5.4 (2)
C-5	0.5561 (9)	0.7714 (9)	0.7635 (5)	4.7 (1)
C-6	0.602 (1)	0.9600 (9)	0.7081 (6)	5.2 (2)
C-7	0.680 (1)	0.9321 (9)	0.5958 (5)	5.1 (2)
C-8	0.5040 (9)	0.7431 (8)	0.5232 (5)	4.2 (1)
C-9	0.4359 (9)	0.5545 (8)	0.5854 (5)	4.0 (1)
C-10	0.3573 (9)	0.5746 (8)	0.6996 (5)	4.4 (1)
C-11	0.277 (1)	0.3565 (9)	0.5106 (5)	5.0 (2)
C-12	0.406 (1)	0.317 (1)	0.4170 (6)	5.4 (2)
C-13	0.4926 (9)	0.4950 (9)	0.3473 (6)	4.4 (1)
C-14	0.6284 (9)	0.6993 (9)	0.4240 (5)	4.2 (1)
C-15	0.728 (1)	0.883 (1)	0.3607 (6)	6.2 (2)
C-16	0.834 (1)	0.835 (1)	0.2626 (6)	6.4 (2)
C-17	0.811 (1)	0.651 (1)	0.2229 (5)	5.6 (2)
C-18	0.671 (1)	0.458 (1)	0.2724 (5)	4.9 (2)
C-19	0.548 (1)	0.278 (1)	0.1869 (6)	6.2 (2)
C-20	0.938 (1)	0.628 (1)	0.1286 (6)	7.4 (3)
C-21	0.774 (1)	0.977 (1)	0.9364 (6)	7.1 (2)
C-22	0.348 (1)	0.871 (1)	0.9215 (7)	6.7 (2)
C-23	0.116 (1)	0.580 (1)	0.7004 (6)	5.8 (2)
C-24	0.298 (1)	0.790 (1)	0.4942 (6)	5.7 (2)
C-25	0.295 (1)	0.496 (1)	0.2803 (6)	5.8 (2)

[a]O-1 position was fixed to define the origin.

TABLE 4. Bond Lengths (Å) and Bond Angles (°) for **2a**.

O-1–C-19	1.221 (7)	C-5–C-10	1.577 (7)	C-12–C-13	1.56 (1)
O-2–C-20	1.21 (1)	C-6–C-7	1.53 (1)	C-13–C-14	1.563 (7)
C-1–C-2	1.54 (1)	C-7–C-8	1.539 (7)	C-13–C-18	1.57 (1)
C-1–C-10	1.52 (1)	C-8–C-9	1.564 (9)	C-13–C-25	1.52 (1)
C-2–C-3	1.513 (9)	C-8–C-14	1.559 (9)	C-14–C-15	1.54 (1)
C-3–C-4	1.50 (1)	C-8–C-24	1.53 (1)	C-15–C-16	1.49 (1)
C-4–C-5	1.55 (1)	C-9–C-10	1.545 (9)	C-16–C-17	1.31 (1)
C-4–C-21	1.551 (9)	C-9–C-11	1.556 (7)	C-17–C-18	1.53 (1)
C-4–C-22	1.55 (1)	C-10–C-23	1.55 (1)	C-17–C-20	1.48 (1)
C-5–C-6	1.53 (1)	C-11–C-12	1.51 (1)	C-18–C-19	1.510 (8)
C-2–C-1–C-10	113.8 (6)	C-9–C-8–C-14	106.9 (5)	C-14–C-13–C-18	107.3 (4)
C-1–C-2–C-3	112.0 (6)	C-9–C-8–C-24	113.4 (4)	C-14–C-13–C-25	114.2 (6)
C-2–C-3–C-4	114.2 (7)	C-14–C-8–C-24	112.9 (6)	C-18–C-13–C-25	109.4 (6)
C-3–C-4–C-5	109.0 (6)	C-8–C-9–C-10	118.3 (5)	C-8–C-14–C-13	117.0 (4)
C-3–C-4–C-21	107.1 (7)	C-8–C-9–C-11	108.9 (5)	C-8–C-14–C-15	112.6 (6)
C-3–C-4–C-22	110.6 (6)	C-10–C-9–C-11	115.4 (4)	C-13–C-14–C-15	110.6 (5)
C-5–C-4–C-21	109.2 (5)	C-1–C-10–C-5	107.2 (5)	C-14–C-15–C-16	112.1 (7)
C-5–C-4–C-22	113.9 (6)	C-1–C-10–C-9	109.8 (5)	C-15–C-16–C-17	124.8 (7)
C-21–C-4–C-22	106.9 (6)	C-1–C-10–C-23	107.5 (5)	C-16–C-17–C-18	123.7 (7)
C-4–C-5–C-6	115.4 (5)	C-5–C-10–C-9	106.4 (4)	C-16–C-17–C-20	118.9 (7)
C-4–C-5–C-10	116.8 (5)	C-5–C-10–C-23	114.0 (5)	C-18–C-17–C-20	117.3 (7)
C-6–C-5–C-10	110.3 (5)	C-9–C-10–C-23	111.8 (6)	C-13–C-18–C-17	110.3 (6)
C-5–C-6–C-7	111.2 (6)	C-9–C-11–C-12	110.3 (4)	C-13–C-18–C-19	110.4 (5)
C-6–C-7–C-8	113.8 (5)	C-11–C-12–C-13	113.4 (6)	C-17–C-18–C-19	110.6 (5)
C-7–C-8–C-9	107.4 (5)	C-12–C-13–C-14	107.1 (5)	O-1–C-19–C-18	122.8 (6)
C-7–C-8–C-14	108.3 (4)	C-12–C-13–C-18	106.9 (6)	O-2–C-20–C-17	125.3 (8)
C-7–C-8–C-24	107.8 (5)	C-12–C-13–C-25	111.6 (5)		

2–C-20–C-17–C-16=178.9(9)°], whereas the intramolecular interactions with the neighboring substituents are responsible for the conformation of the other aldehyde group [O-1–C-19–C-18–C-13=−86.1(7)°].

The molecule shows a remarkable bend, normal to the average molecular plane, which can be quantified by an angle of about 30° between the best planes of the *A* and *C* rings and corresponds to a radius of curvature of about 10 Å. As the crystal packing, shown in Figure 2, is governed by normal Van der Waals interactions, the accentuated bend of the molecule is probably determined by the strong intramolecular interactions between the methyl substituents along the "spine" of the molecule. The shortest intermolecular interactions are O-2·····$C\text{-}25_{(1+x,y,z)}$=3.29(1) Å, O-1·····$C\text{-}15_{(x,y-1,z)}$=3.37(1) Å and C-20·····$C\text{-}25_{(1+x,y,z)}$=3.46(1) Å.

It is well known that sesquiterpenoid 1,4-dialdehydes, such as polygodial [**4**], with suitable distances between the two aldehyde groups and an adjacent intracycle unsaturation, exhibit antifeedant activity and a hot taste for humans (10). However, the scalaradial molecule [**2b**], which embodies all these structural features, is tasteless (10) and displays the same antifeeding effects on fish of polygodial, but at twice the concentration of **4** (12). These effects were ascribed to the larger size of scalaradial.

On the other hand, the biological response of 12-deacetoxyscalaradial [**2a**] is similar to that reported for polygodial (10,12). In fact, on comparing the activity of **2a** and **2b**, the two compounds were active in the fish feeding inhibition bioassay at a concentration of 30 and 60 $\mu g/cm^2$, respectively and only **2a** was hot to the taste. These results show that molecular size, as such, is not a restrictive factor in these activities, but point out the specific importance of the substituent at C-12 in **2a** and **2b**, or in the equivalent C-1 position of a supposed polygodial derivative. The presence of a bulky substituent in this position, such as the acetoxy group in **2b**, may inhibit the biological activity of the metabolite, either by altering the conformation of the nearby aldehyde group, or altering the surface complementarity between the molecule and the binding site of the receptor.

Both compounds **2a** and **2b** are cytotoxic (LD_{50}=0.77 μg/ml and LD_{50}=0.18 μg/ml for **2a** and **2b** respectively) in the *Artemia salina* shrimp bioassay (13).

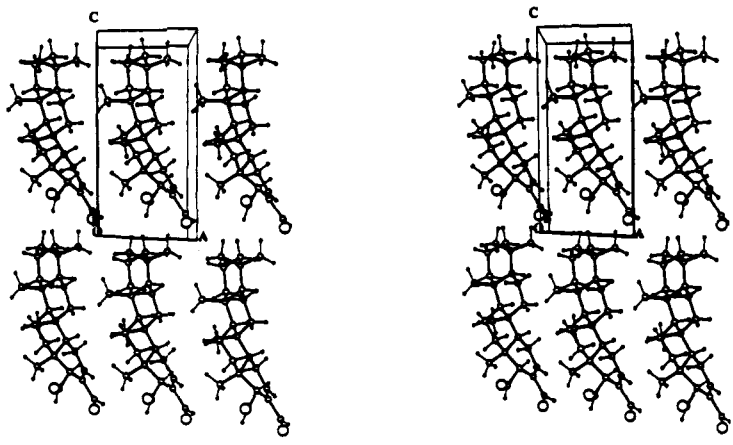

FIGURE 2. Stereoview of the Crystal Packing of **2a**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were measured on a Kofler apparatus and are uncorrected. Uv spectra were obtained on a Varian DMS 90 spectrophotometer. Ir spectra were recorded on a Bio-Rad FTS-7 FT-IR spectrometer. Optical rotations were measured on a Jasco DIP 370 polarimeter, using a 10-cm microcell. Mass spectra were recorded on an Fisons TRIO 2000 spectrometer, coupled with an INTEL computer. ^{1}H-nmr and ^{13}C-nmr spectra were recorded at 500 and 125 MHz, respectively, with TMS as internal standard on a Bruker AM 500 instrument, under Aspect X32 control. The 2D nmr spectra were obtained using Bruker's microprograms. Si gel chromatography was performed using pre-coated Merck F_{254} plates and Merck Kieselgel 60 powder. For the crystallographic work, an Enraf-Nonius CAD-4F diffractometer, SDP software and MicroVax 3100 Digital computer were used.

ANIMAL MATERIAL.—*Cacospongia mollior* was collected by hand at about 5 m depth in the Bay of Naples in September 1991 and March 1992, and frozen at −20° until extracted. A voucher specimen is maintained in the institute collection (voucher No. S6C).

EXTRACTION AND ISOLATION.—The frozen sponge (200 g dry wt after extraction) was extracted with Me_2CO and, after elimination of the solvent *in vacuo*, the aqueous residue was extracted with Et_2O and then with *n*-BuOH. The Et_2O extract was evaporated *in vacuo* to obtain a brown oil (13 g), which was applied to a column of Si gel. The column was eluted with a solvent gradient system from petroleum ether (40–70°) to Et_2O.

Fractions with the same tlc profile were combined. Three fractions with scalarane sesterterpenoids were recovered. The least polar fraction after crystallization from EtOH, gave **2a** (18 mg), while **3** (420 mg) and **2b** (1.96 g) were recovered from other fractions, after crystallization from EtOH.

12-Deacetoxyscalaradial [**2a**].—Mp 200–203° (EtOH); $[\alpha]^{25}D$ −19° (*c*=0.7, $CHCl_3$); uv λ max (MeOH) 231 (ε 14000) nm; ir ν max ($CHCl_3$) 2845, 1725, 1680, 1640 cm^{-1}; eims *m/z* $[M]^+$ 370 (8), 343 (24), 342 (88), 191 (100), 189 (25), 121 (45); nmr data, see Table 1.

X-RAY STRUCTURE DETERMINATION.—Compound **2a** crystallized from EtOH in the form of irregular plates and a single crystal of size 0.35×0.31×0.06 mm was selected for the crystallographic study. Accurate cell parameters were obtained by least-squares refinement of the setting angles of 25 reflections at medium θ (20°<θ<24°), using graphite monochromated CuKα radiation and an Enraf-Nonius CAD-4F diffractometer.

Crystal data[1].—$C_{25}H_{38}O_2$, MW=370.58, triclinic, space group P_1 with *a*=6.361(1), *b*=7.214(2), *c*=12.691(3) Å, α=97.17(2), β=90.31(2), γ=114.44(1)°, V=525.0(4) Å^3, *Z*=1, Dc=1.172g·cm^{-3}.

Intensities of 2087 independent reflections (θ max =73°) were collected at room temperature, using ω scan mode, as suggested by peak-shape analysis, and about 2° of scans, required by the wide angular spread

[1]Structure factors, hydrogen atom parameters, and isotropic thermal parameters have been deposited with the Cambridge Crystallographic Data Centre and can be obtained from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, UK.

of reflections, which denotes accentuated mosaicity in the crystals. During the data collection the intensities of four standard reflections were monitored every 4 h (4% variation) in order to check the crystal and equipment stability. No significant intensity decay was observed. The intensities were corrected for Lorentz and polarization factors, but not for the absorption effect ($\mu=5.17$ cm^{-1}). The structure was solved using MULTAN82 (14) and subsequent difference Fourier syntheses. The positional and anisotropic temperature parameters for non-hydrogen atoms were refined by full-matrix (on F) least squares method, the coordinates of O-1 were held fixed to define the origin. At convergence H-atoms were generated at the expected positions with a C-H distance 1.03 Å, taking into account for methyl groups the indications from ΔF map. All hydrogens were included in the last refinement, as fixed atoms and with isotropic thermal parameters set equal to Beq of parent atoms.

At convergence, the final discrepancy index $R=\Sigma(|Fo|-|Fc|)/\Sigma|Fo|$ was 0.067 for 1419 observed [$I\geq 2.5\sigma(I)$] reflections and 242 refined parameters, including a correction factor for secondary extinction (15) equal to $3.7(8)\times10^{-6}$; Rw=0.067, with $w=1/\sigma^2(Fo)+(0.02Fc)^2+0.5$ (16). The maximum and minimum values in the final difference Fourier synthesis are 0.19 and 0.20 eÅ^{-3}, respectively. For the crystallographic work, Enraf-Nonius SDP software (17) and a MicroVax 3100 computer were used.

BIOLOGICAL EVALUATIONS.—*Human taste bioassay.*—Filter paper disks 1 cm in diameter were dipped into 0.5%, and 1% Me_2CO solutions of the dialdehydes, air-dried, and then tasted for hotness by a group of four people. Compound **2a** has a hot taste at both concentrations, while **2b** is tasteless.

Feeding inhibition bioassay.—The fresh water fish was *Carassius carassius*, used in feeding inhibition bioassays as already described (12), giving a response at the minimum active concentration of 30 and 60 μg/cm^2 for **2a** and **2b** respectively.

Brine shrimp lethality.—The brine shrimp lethality assay performed as described by Meyer *et al.* (13), gave **2a** $LD_{50}=0.77$ μg/ml, **2b**, $LD_{50}=0.18$ μg/ml.

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