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C-16 Hydroxylated Abietane Diterpenes from *Salvia mellifera*. Absolute Configuration and Biogenetic Implications.

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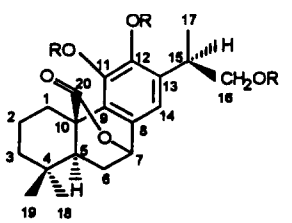
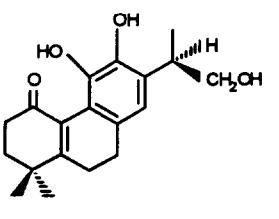
Abstract **Two new C-16 hydroxylated abietane diterpenes and the known compounds cryptotanshinone and isopimaradiene have now been isolated from *Salvia mellifera* and identified by rigorous spectroscopic and X-ray crystallographic analyses. Our findings indicate that epoxidation of the monosubstituted double bond of an isopimaradiene precursor must take place before 16-hydroxylated abietanes are formed.**

Salvia species (Labiatae) figure prominently in the pharmacopoeias of many countries throughout the world[1] and are also one of the most widespread genus with over 500 species. One of the best known examples is *Salvia miltiorrhiza*, Chinese sage, from the roots of which many abietanoid diterpenes have been isolated. These diterpenes are thought to be the active components of the medicines prepared from this species[2].

Salvia mellifera Greene[3] grows abundantly in California below 2000 ft. The fresh and dried leaves of *S. mellifera* have been shown to possess a wide range of ethnomedical properties[4]. We have already[5] reported the isolation of the new diterpenes 11,12-dihydroxy-20-nor-abieta-5(10),8,11,13-tetraen-1-one and isogaldosol together with the known compounds carnosic acid, carnosol, rosmanol, rosmadial, galdosol and isorosmanol from the aerial part of the plant. The co-occurrence of all these compounds in one species taken in conjunction with their chemical behaviour led to the postulation of a biogenetic pathway[5] to highly oxidized abietatriene diterpenes in which enzymatic dehydrogenation processes and the participation of singlet-state oxygen appear to play an important role. Further examination of the same extract has led to the isolation of two new C-16 hydroxylated abietane diterpenes, **1** and **3** and the known compounds cryptotanshinone (**4**) and isopimaradiene (**5**).



1 R=H
2 R=Ac



3

The appearance of 20 carbons in the ^{13}C NMR spectra of **1** (see Table 1) and the high resolution mass spectrum which showed a molecular ion at m/z 346.1738 for the molecular formula $C_{20}H_{26}O_5$ and therefore eight units of unsaturation suggested a diterpene with a trisubstituted diphenolic ring (δ 144.01, 143.26, 132.63, 129.38 and 122.25, s; 113.35 d) and a lactone group (^{13}C NMR δ: 176.67; IR, 1722 cm^{-1}).

Table 1.- ^{13}C NMR Chemical Shifts of **1** in $CDCl_3$.

C	1	C	1
1	29.17 (t)	11	144.01 (s)
2	19.26 (t)	12	143.26 (s)
3	41.49 (t)	13	129.38 (s)
4	34.90 (s)	14	113.35 (d)
5	45.87 (d)	15	37.87 (d)
6	30.15 (t)	16	70.48 (t)
7	78.15 (d)	17	15.48 (q)
8	132.63 (s)	18	20.08 (q)
9	122.25 (s)	19	32.14 (q)
10	48.77 (s)	20	176.67 (s)

The appearance of a monohydroxylated isopropyl group as one of the aromatic ring substituents as suggested by the downfield shift of the methine proton (^1H NMR δ: 1.33, d, J=7.3 Hz; 3.14, sext, J=7.3 Hz; 3.76, t, J=9.3 Hz; 4.02, dd, J_1=9.3, J_2=3.0 Hz; ^{13}C NMR δ: 15.48, q; 37.87, d; 70.48, t) was highly reminiscent of a dehydroabietane skeleton. The COSY (homonuclear correlation) spectrum which confirmed the latter grouping and HETCOR spectrum (one-bond ^1H-^{13}C heteronuclear correlation) enabled the two isolated spin systems of C-1 to C-3 (three sequential methylene groups) and C-5 to C-7 (a methine, a methylene and a methine in sequence) to be delineated.

Acetylation of **1** gave the triacetate **2** ($C_{26}H_{32}O_8$, HRMS) with ^1H NMR signals for the methyls of two aromatic and one aliphatic acetate as singlets at δ 2.32, 2.29 and 2.03, respectively. The fifth oxygen atom of **1** therefore belonged to the ether bridge of the lactone ring. The downfield shift of both C-7 and H-7 (δ 78.15, d; 5.35, dd, J_1=4.0, J_2=1.0 Hz, respectively) fixed the position of the lactone closure.

A ROE (see Table 2) between H-7 and the aromatic proton (^1H NMR δ 6.51, s) situated the latter on C-14. Furthermore a FLOCK spectrum[6] (for long-range ^1H-^{13}C heteronuclear scalar correlation) which showed three-bond coupling from H-14 to C-15 established the position of the monohydroxylated isopropyl group at C-13 (see Table 3). This experiment also showed three-bond coupling from H-18 to C-19 and from H-19 to C-18 confirming the geminal nature of the methyl singlets in the ^1H NMR spectrum (the one-bond couplings were originally assigned from the HETCOR spectrum). Long-range heteronuclear couplings also to be seen in this spectrum indicated that the *gem*-dimethyl group connected the two spin systems (C-1 through C-3 and C-5 through C-7) by couplings between both H-18 and H-19 with C-3, C-4 and C-5.

Table 2.- ^{1}H NOE for 1

Position	^{1}H NOE*	Position	^{1}H NOE*
H-19	H-2β, H-6β	H-7	H-6α, H-6β
H-18	H-6α, H-6β	H-5	H-1α, H-6α H-6β, H-18
H-17	H-14, H-15 H-16, H-16'	H-1α	H-1β, H-3α
H-16, H-16'	H-15	H-1β	H-2β, H-2α
H-14	H-7, H-15		

* From a ROESY experiment run in $CDCl_3$.

Tabla 3.- ^{13}C-^{1}H Long-Range Couplings for 1 in $CDCl_3$.

^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
H-16 H-16'	C-13 C-17	H-15	C-1, C-4 C-9, C-10 C-18, C-19 C-20	H-1α	C-3, C-9 C-10, C-20
H-14	C-7, C-9 C-12, C-15	H-6β	C-7, C-8 C-10		
H-7	C-5, C-9 C-14, C-20	H-2α	C-4, C-10		

These observations indicated the typical abietane nature of the A and B rings with the angular C-20 oxidized to a lactone carbonyl (δ 175.5), and established the structure of 1 as that of 16-hydroxycarnosol. The location of the lactone carbonyl at C-20 was supported by long-range heteronuclear couplings to this carbonyl carbon (δ 175.5) from H-1α and H-5 observed in the cross-section at the C-20 resonance frequency of the FLOCK spectrum. A ROE between H-5 and H-1α confirmed the axial orientation of H-5 (relative to the B ring) and hence the *trans* stereochemistry of the AB ring fusion, as expected.

Compound 1 gave suitable crystals for X-ray structural analysis which established that its configuration was as shown in Figure 1 with the following stereochemistry: C-5/H-5α, C-7/H-7α, C-5S, C-7S, C-15R. The six-membered ring (C-1, C-2, C-3, C-4, C-5, C-10) had a chair conformation although this was slightly flattened as the average torsion angle was 52°; the other six-membered rings adopted boat conformations with a flap at C-7/C-10. The ring junction between Rings (C-1, C-2, C-3, C-4, C-5, C-10)/(C-9, C-8, C-7, C-6, C-5, C-10) was *trans*.

The molecules in the crystal were linked by hydrogen bonds and there were two intramolecular H-bonds between O-1,O-2 and O-2,O-3 and an intermolecular one between O-1,O-4. The geometrical details of the H-bonds and symmetry codes were as follows:

X-H.....Y	X-H (Å)	X.....Y (Å)	H.....Y (Å)	<X-H.....Y (°)
(1) O-1-H.....O-4	0.83 (6)	2.684 (3)	1.87 (6)	168.2 (5.6)
(0) O-2-H.....O-1	0.90 (4)	2.541 (3)	1.64 (4)	171.1 (4.1)
(0) O-3-H.....O-2	0.95 (4)	2.581 (3)	2.01 (4)	116.4 (2.7)

(1) X, Y, 1+Z

(0) X, Y, Z

Figure 2 shows the molecular arrangement consisting of layers of molecules parallel to an axis. Some other contacts less than 3.5 (Å) were also perceived.

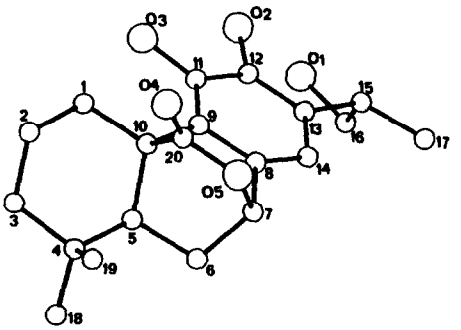


Fig. 1.- PLUTO[16] drawing of the molecule with the atomic numbering.

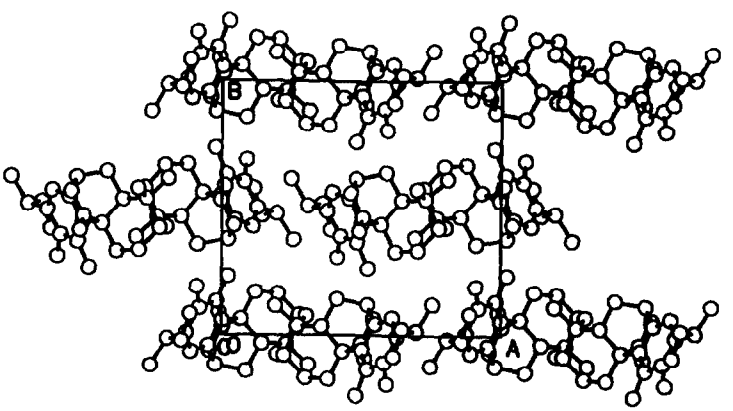


Fig. 2.- The packing arrangement seen down c axis drawn by PLUTO[16].

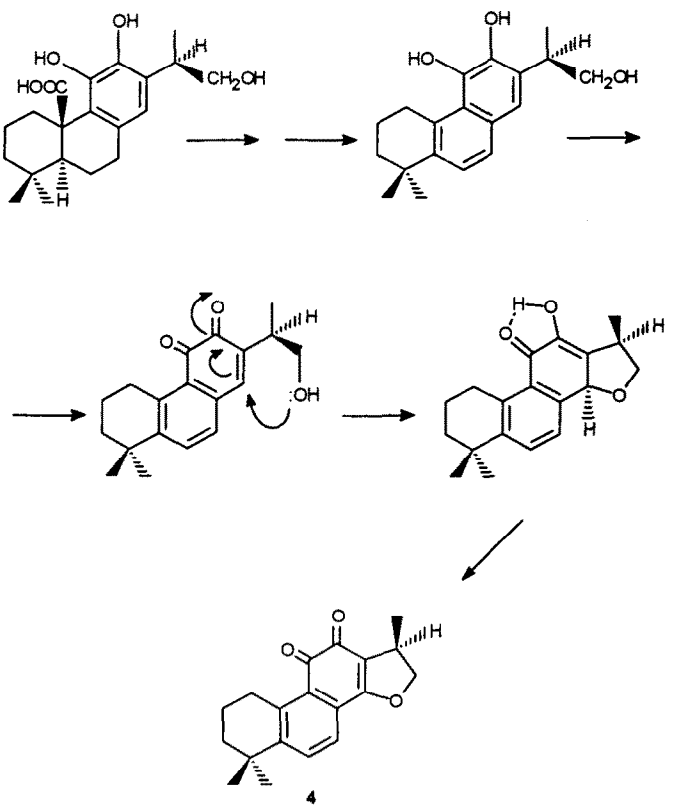
The structure of **3** was established as 11,12,16-trihydroxy-20-nor-abieta-5(10),8,11,13-tetraen-1-one as follows. High resolution mass spectrum showed a molecular ion at m/z 316.16741 requiring a molecular formula of $C_{19}H_{24}O_4$ and therefore eight unsaturation units. The IR spectrum had bands for phenols (3455 cm^{-1}), an alcohol (3300 cm^{-1}) and an unsaturated ketone (1635 cm^{-1}), findings wich were confirmed by the UV spectrum (λ_{max} 318 and 286 nm). In the low-field region of the ^1H NMR spectrum, two protons at δ 9.52 and 6.27, interchangeable with deuterium oxide, could be assigned to the phenolic hydroxy groups and one aromatic proton appeared as a singlet at δ 6.58. Signals were observed for only two angular methyls and the presence of the monohydroxylated isopropyl group was established by the appearance of three-proton doublet

at δ 1.28, (J= 7.0 Hz), a one-proton sextuplet at δ 3.39 (J= 7.0 Hz) and a two-proton doublet at δ 3.77 (J= 6.0 Hz) and was confirmed by the fragmentation m/z 257 [M^+-C_3H_7O] in MS. No vinyl proton was visible in the ^1H NMR spectrum.

The above data indicated that **3** was a $\Delta^{5,10}$-nor-abietane-hydroxy-diphenol with a carbonyl group on C-1 or C-6. Double resonance experiments using high-field ^1H NMR demonstrated the presence of an A_2X_2 system as two-proton triplets centred al δ 2.71 and 1.96 (J=7.0 Hz) and an A_2B_2 system as two-proton multiplets at δ 2.42 and 2.38. Irradiation of the aromatic proton gave a NOE effect with the B_2 part of the A_2B_2 system. These facts established the position of the aromatic proton on C-14 and the ketone group on C-1. Biogenentic considerations assigned **3** the same 15R configuration as **1**.

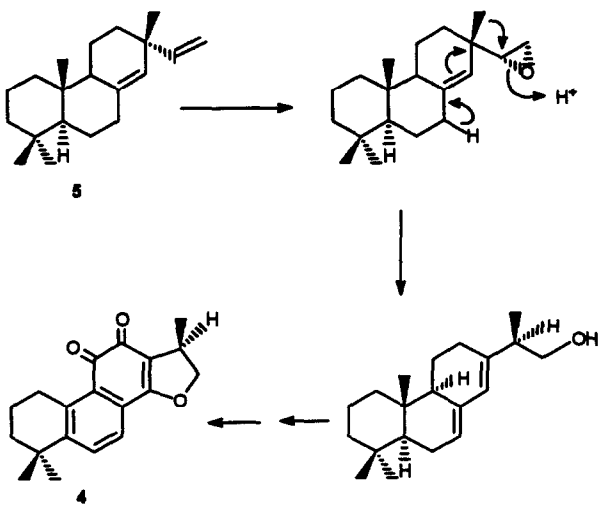
Compound **4** isolated from a root extract was identified from its spectral data as cryptotanshinone[7] with the same $[\alpha]_D^{20}$= -92.3° as that of crypotanshinone isolated from *S. miltiorrhiza*[7] which has an unequivocal 15R absolute configuration[8] founded on biosynthetic studies. These observations agreed with our proposed biogenetic route to cryptotanshinone from 16-hydroxy carnosic acid (Scheme 1) since the configuration at C-15 would not be involved in the formation of the dihydrofuran ring.

Scheme 1



It has already been demonstrated that in the biosynthesis of cryptotanshinone[8] and ferruginol[9], the abietane skeleton is formed by 1,2-methyl migration from C-13 to C-15 on the si-face of the double bond in the 8(14),15-isopimaradiene precursor (**5**), also isolated from this extract of *S. mellifera* with spectral data superimposable on those given in the literature[10]. This methyl becomes the *pro* (R) methyl in the isopropyl group in ferruginol and would seem to be a general biosynthetic feature of the formation of abibetane skeletons in the genus *Salvia*. The formation of C-16 hydroxylated abietanes must therefore involve prior epoxidation of the monosubstituted double bond of isopimaradiene (Scheme 2).

Scheme 2



EXPERIMENTAL

General.- ^1H and ^{13}C NMR spectra were recorded on Bruker AMX400 and WP200SY spectrometers, IR spectra were taken on a Perkin-Elmer 1600 (FTIR) spectrophotometer and UV spectra on a Perkin-Elmer 550SE instrument. High resolution mass spectra were run on a VG-Micromass ZAB-2F at 70 eV.

Isolation of Products.- *S. mellifera* Greene was collected on the hillsides of Bluebird Canyon Road, Laguna Beach, California in August 1988 and a voucher specimen is on file in the Musseum of Systematic Biology, University of California, Irvine. The dried, ground stems and leaves (934 g) were extracted with distilled Me_2CO at room temperature and the solvent eliminated under reduced pressure at 40°C, giving an extract (138 g) which was subjected to flash chromatography on silica gel with mixtures of hexane/ethyl ether and hexane-EtOAc of increasing polarity. The fraction eluted with 50% EtOAc contained **1** and that with 80% ethyl ether/n-hexane, compound **3**. Both fractions were purified by chromatography on Sephadex LH-20 with 2:1:1 n-hexane/$CHCl_3$/MeOH as eluant and then by preparative TLC with 98:2 CH_2Cl_2/acetone to give **1** and **3**.

16-Hydroxycarnosol (1).- (300 mg) was isolated as a white crystalline solid; $[M]^+$at m/z 346.1738 (calc. for $C_{20}H_{26}O_5$, 346.1696); $[\alpha]_D$= -72.39° ($CHCl_3$; c 0.384); UV λ_{max} (EtOH) nm: 283; IR ν_{max} cm^{-1}: 3469, 2953, 1722, 1456, 1393, 1335, 1299, 1249, 1218, 1160, 1122, 1070, 1028, 987, 947, 918, 863, 831; ^1H NMR (400 MHz, $CDCl_3$) δ: 0.86 (3H, s, Me-19), 0.91 (3H, s, Me-18), 1.33 (3H, d, J= 7.3 Hz, Me-17), 1.76-1.94 (complex, H-3β, H-2α, H-3α, H-5 and H-2β), 2.20 (1H, sext, H-6β), 2.50 (1H, td, H-1α), 2.89 (1H, br d, J= 13.4 Hz, H-1β), 3.14 (1H, sext, J= 7.3 Hz, H-15), 3.76 (1H, t, J= 9.3 Hz, H-16), 4.02 (1H, dd, J_1= 3.0, J_2= 9.3 Hz, H-16), 5.34, 5.36 (1H, dd, J_1= 1.0, J_2= 4.0 Hz, H-7), 6.23 (1H, s, Ar-O<u>H</u>, C-11), 6.51 (1H, s, H-14), 8.80 (1H, br s, Ar-O<u>H</u>, C-12); ^{13}C NMR (20 MHz, $CDCl_3$), see Table 1; EIMS (rel. int) m/z: 346 $[M]^+$ (38), 316 (14), 302 (100), 284 (53), 271 (22), 231 (14), 213 (35), 201 (14), 149 (10), 136 (16), 128 (12), 91 (62), 85 (10), 65 (15), 55 (27).

Crystal Data of 1.- $C_{20}H_{26}O_5$, MS 346.1738, orthorhombic symmetry, space group $P2_12_12_1$, cell dimensions: a= 13.546 (1), b= 12.258 (1), c= 10.783 (1) Å, V= 1790.4 (3) $Å^3$, Z= 4, F(000)= 744.0, Dx= 1.2851 mg^{-3}, μ= 7.071 cm^{-1}. Cell parameters and estimated standard deviations were calculated from setting angles of 51 reflections 10°<2Θ<76°, crystal dimension 0.40 x 0.32 x 0.39 mm.

The lattice parameters and intensities were measured on a Philips PW 1100 four-circle diffractometer using ω/2Θ scan mode with ω scan width 1.60, ##(>w##) B scan speed 1 deg/min and with graphite monochromated CuKα (λ = 1.5418 Å) radiation. A total of 1758 independent reflections were collected in the range 2°<Θ<65° giving 1702 reflections did not show any significant change in their intensities during the course of the experiement. The intensities were corrected for Lorentz and polarization effects but not for absorption.

The structure was solved by direct methods (SIR 88)[11] and Fourier difference synthesis. All non-H atoms were refined anisotropically using full-matrix least-squares refinement. H-atoms were subsequently located in Δρ maps, and were included in the last cycles of refinement, and refined. A weighting scheme was not used to give dependence in <wΔ²F> vs. <Fo> and <sinΘ/λ>. Final R and Rw values were 3.4 and 3.7, respectively. The maximum residual electron density was 0.18 $eÅ^{-3}$.

The absolute configuration was determined by the Bijvoet method[12] and η-refinement for the oxygen dispersors[13]. On considering reflections with Fo>10σ(Fo) there are 29 Friedel pairs with Fc>10.0 and less experimental error, showing an averaged Bijvoet difference of 1.028 for the (Δ +) right enantiomer vs. 1.030 for the wrong one (Δ -).

The η-refinements were made at (+x, +y, +z) Δf''= 0.00 without averaging I's. The refinements converged to η values of +0.028 (9) which confirms the absolute configuration of the molecule. Atomic scattering factors and anomalous dispersion corrections followed the conventions given in the literature[14]. All calculations were made on a VAX 6410 computer using the XRAY-76 system[15]. Tables of atomic parameters, bond lengths, bond angles and torsion angles are provided as supplementary material.

Acetylation of 1.- Compound **1** (2.9 mg) was dissolved in pyridine and twice the volume of acetic anhydride was added to afford a triacetyl derivative (1.8 mg) after purification by chromatography.

11,12,16-Triacetoxycarnosol (2).- $[M]^+$ at m/z 472.2098 (calc. for $c_{26}H_{32}O_8$, 472.2096); ^1H NMR (200 MHz, $CDCl_3$) δ: 0.88 (3H, s, Me-19), 0.92 (3H, s, Me-18), 1.25 (3H, d, J= 7.0 Hz, Me-17), 2.03 (3H, s, OAc-16), 2.29 (3H, s, Ac-O<u>Ac</u>), 2.32 (3H, s, Ac-O<u>Ac</u>), 2.65 (1H, br d, J= 14.0 Hz, H-1β), 3.25 (1H, sext,

J= 7.0 Hz, H-15), 4.07 (2H, m, H-16), 5.50 (1H, dd, J_1= 1.0, J_2= 4.0 Hz, H-7), 7.10 (1H, s, H-14); ^{1}H NMR (200 MHz, Be-d_6) δ: 0.50 (3H, s, Me-19), 0.90 (3H, s, Me-18), 0.98 (3H, d, J= 7.0 Hz, Me-17), 1.64 (3H, s, Ac-OAc), 1.72 (3H, s, Ac-OAc), 1.89 (3H, s, OAc-16), 2.94 (1H, br d, J= 14.0 Hz, H-1β), 3.37 (1H, sext, H-15), 4.06 (2H, m, H-16), 4.93 (1H, dd, J_1= 1.0, J_2= 4.0 Hz, H-7), 6.64 (1H, s, H-14); EIMS (rel. int) m/z: 472 $[M]^+$ (3), 388 (33), 342 (100), 284 (39), 282 (37), 273 (12), 241 (6), 213 (80), 199 (19), 185 (15), 165 (22), 155 (25), 141 (40), 128 (52), 115 (48), 91 (25), 69 (41), 55 (52).

11,12,16-Trihydroxy-20-nor-abieta-5(10),8,11,13-tetraen-1-one (3).- (2.0 mg) was isolated as an amorphous solid; $[M]^+$ at m/z 316.16741 (calc. for $C_{19}H_{24}O_4$, 316.16746); UV λ_{max} (EtOH) nm: 286, 232; IR ν_{max} cm^{-1}: 3455, 2958, 2927, 2866, 1739, 1635, 1441, 1383, 1279, 1118, 1069, 1031; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.28 (3H, d, J= 7.0 Hz, Me-17), 1.29 (6H, s, Me-18, Me-19), 1.96 (2H, t, J= 7.0 Hz, H-3), 2.38 (2H, m, H-6), 2.42 (2H, m, H-7), 2.71 (2H, t, J= 7.0 Hz, H-2), 3.39 (1H, sext, H-15), 3.77 (2H, d, J= 6.0 Hz, H-16), 6.27 (1H, br, s, ArOH), 6.58 (1H, s, H-14), 9.52 (1H, s, ArOH); EIMS (rel. int) m/z: 316 $[M]^+$ (50), 286 (21), 285 (100), 257 (9), 242 (4), 225 (4), 211 (4), 199 (4), 195 (5), 183 (5), 169 (6), 153 (5), 141 (7), 128 (7), 115 (8), 91 (7), 83 (9), 69 (18), 59 (24), 55 (27).

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