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# ANTIMICROBIAL AND ANTISPASMODIC TETRAHYDROANTHRACENES FROM *CASSIA SINGUEANA*

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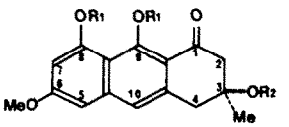
**Abstract** Four tetrahydroanthracene derivatives with antimicrobial and antispasmodic activities have been isolated from *Cassia singueana*. The evidence described in the following indicates them to be torosachrysone (**1**), germichrysone (**4**), and two new dimeric tetrahydroanthracenes, singueanol-I (**7**) or 6,6′-dimethoxy-3,3′,8,8′,9,9′-hexahydroxy-3,3′,7,7′-tetramethyl-3,3′,4,4′-tetrahydro(10,10′-bianthracen)-1,1′(2H,2′H)-dione, and singueanol-II (**8**) or 6,6′-dimethoxy-3,3′,8,8′,9,9′-hexahydroxy-3,3′,7,7′-tetramethyl-3,3′,4,4′-tetrahydro(5,10′-bianthracen)-1,1′(2H,2′H)-dione.

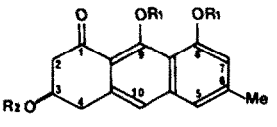
Tetrahydroanthracenes have been isolated from a wide variety of sources, e.g. aloesaponel-I and aloesaponol-II from *Aloe saponaria*,[1] julichromes from *Streptmyces shiodensis*,[2] phlegmacin from *Cortinarius odorifer*,[3,4] and *Cassia torosa*,[5] flavomannin from *Penicillium wortmanni*,[6,7] and tetrahydroanthracenes from *Karwinskia humboldtiana*.[8] Torosachrysone (**1**)[9] and germichrysone (**4**)[10] discussed in this report have been isolated from seedlings of *Cassia torosa*. Although the crystal structure of germichrysone (**4**) has been determined by the X-ray Bijvoet method,[11] the C-3 configurations of many tetrahydroanthracenes remain unknown.

Four tetrahydroanthracenes, which were identified to be torosachrysone (**1**), germichrysone (**4**), and two new dimeric tetrahydroanthracenes, singueanol-I (**7**), and singueanol-II (**8**), were isolated from the roots of *Cassia singueana*, an East African medicinal plant. The tetrahydroanthracenes inhibit the growth of gram-positive bacteria, the minimal inhibitory concentrations being as follows; torosachrysone (**1**): $70 \times 10^{-6}$ g/ml (*Staphylococcus aureus*), germichrysone (**4**): $30 \times 10^{-6}$ g/ml (*Corynebacterium equi*), singueanol-I (**7**): $25 \times 10^{-6}$ g/ml (*S. aureus*), and singueanol-II (**8**): $50 \times 10^{-6}$ g/ml (*S. aureus*). Singueanol-I (**7**) also exhibits papaverin-like antispasmodic activity, i.e., it causes relaxation of an isolated guinea pig colon contracted with barium chloride.

The UV spectra of singueanol-I (**7**) and singueanol-II (**8**) (Fig. 4) show that they are dimeric and that the chromophore is similar to that of torosachrysone (**1**). The PMR data of torosachrysone (**1**) were employed as standard references in order to determine the structures of these dimeric tetrahydroanthracenes. A comparison with the PMR chemical shifts of the corresponding protons in torosachrysone (**1**) shows that the 4-H, 4′-H, 5-H, 5′-H, 10-H, 10′-H, 6-OMe, and 6′-OMe signals in singueanol-I (**7**) and singueanol-II (**8**) are located at unusually high fields (Fig. 1). These unusual high field shifts can be ascribed to the naphthalenoid ring current effect which is manifested in the twisted dimeric structures (Fig. 5), a result which is fully supported by the strong split CD curves of the coupled oscilator type. Furthermore, molecular models show that all protons undergoing the high field shifts as compared to the monomer should be located close to the other aromatic moiety linked by the connecting bond.

The PMR data of singueanol-I (**7**, Fig. 1) indicate it to be a symmetric dimer of two tetrahydroanthracene moieties, while the 7,7′-bianthracene structure encountered in flavomannin[6,7] can be eliminated on the grounds described above. The sole aromatic proton peak which appears at the unusually high field of 5.98 ppm is neither coupled to 4-H nor to the aromatic methyl; in contrast, 10-H in torosachrysone (**1**) is coupled to 4-H, and the 5-H/7-H signals in germichrysone (**4**) are coupled to 6-Me. This evidence suggests that the aromatic proton is located at 5 and that the aromatic methyl is not 6-Me, i.e. a position ortho to 5-H. The positioning of the methoxyl group at C-6 is based on its high chemical shift relative to




1 $R_1 = R_2 = H$

2 $R_1 = Me$, $R_2 = H$

3 $R_1 = Me$, $R_2 = Bz$

4 $R_1 = R_2 = H$

5 $R_1 = Me$, $R_2 = H$

6 $R_1 = Me$, $R_2 = Bz$

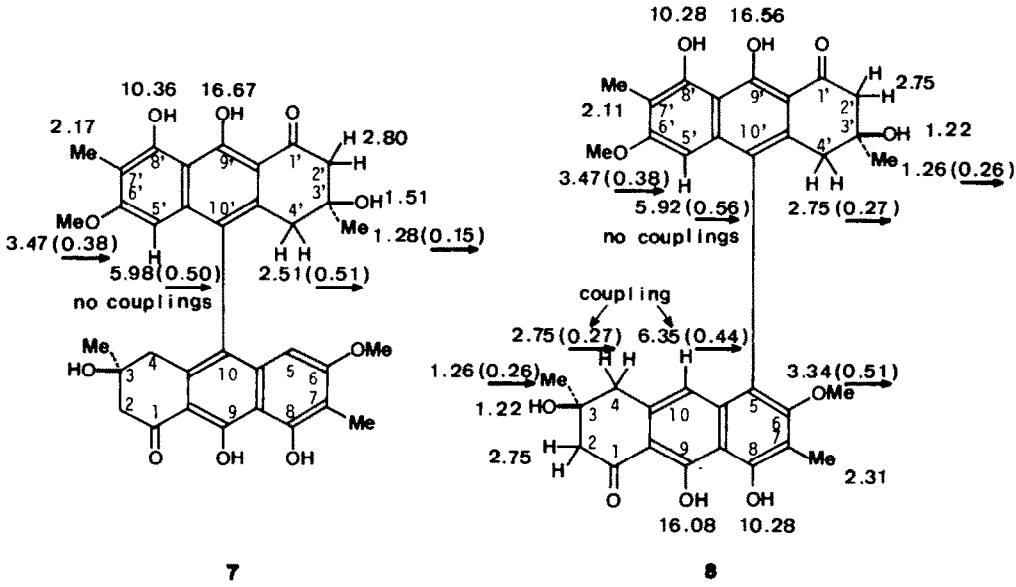



Fig. 1. PMR data of singueanol-I (**7**) and singueanol-II (**8**): 5′-H 5.98 (0.50) denotes that 5′-H appears at 5.98 ppm or at a field 0.50 ppm higher than the corresponding proton signal in torosachrysone (**1**), the reference sample.

torosachrysone (**1**) and the similarities in the UV spectra of singueanol-I (**7**) and torosachrysone (**1**, as described above). Thus the 6,6′-dimethoxy-3,3′,8,8′,9,9′-hexahydroxy-3,3′,7,7′-tetramethyl-3,3′,4,4′-tetrahydro(10,10′-bianthracen)-1,1′(2H,2′H-dione (**7**) is derived for singueanol-I.

The PMR data (Fig. 1) show that singueanol-II (**8**) has an unsymmetric dimer structure. The 7,10′-bianthracene structure encountered in phlegmacin[3-5] can be eliminated because it fails to explain the shifts to high field of the various proton signals shown in structure (**8**, Fig. 1). The aromatic proton at 5.92 ppm is neither coupled to 4-H nor to the aromatic methyl, while another aromatic proton at 6.35 ppm is long-range coupled to 4-H. This leads to the 6,6′-dimethoxy-3,3′,8,8′,9,9′-hexahydroxy-3,3′,7,7′-tetramethyl-3,3′,4,4′-tetrahydro(5,10′-bianthracen)-1,1′(2H,2′H)-dione (**8**) structure for singueanol-II.

It is well known that the CD Cotton effects due to a chiral exciton coupling between two or more chromophores enable one to determine the absolute stereochemistry in a nonempirical manner.[12-15] Harada *et al.*[16] have employed the chiral exciton coupling between the electric transition moments of the benzoate chromophore and the naphthalene chromophore to determine the absolute stereochemistry of chromomycin $A_3$. Consequently torosachrysone (**1**) was converted into its benzoate in order to determine the absolute configuration at C-3. Torosachrysone (**1**) was methylated with diazomethane and benzoylated with benzoyl chloride in pyridine to give the dimethyl ether monobenzoate (**3**). The PMR peaks (Fig. 2) of 3-methyl, 2(equatorial)-H, and 4(equatorial)-H undergo large shifts to lower fields by benzoylation while those of 2(axial)-H and 4(axial)-H are less affected. An axial conformation can be

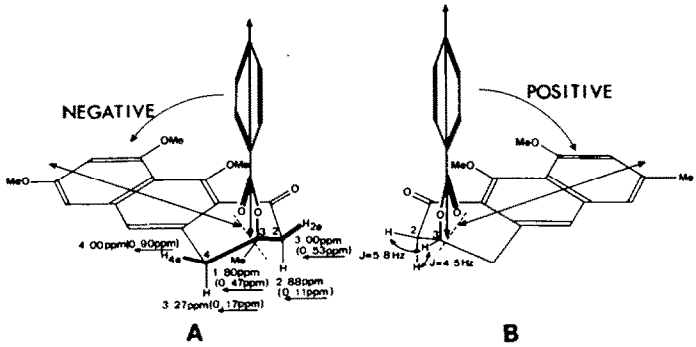



Fig. 2. (A) PMR data of torosachrysone dimethyl ether monobenzoate (**3**); small arrows show the low-field shifts caused by the benzoylation; W-letter type coupling between 2(equatorial)-H and 4(equatorial)-H; stereo view of the negative exciton chirality; long arrows show the long axes of the two naphthalene chromophores. (B) PMR coupling constants between 2-H and 3-H in germichrysone dimethyl ether monobenzoate (**6**); stereo view of the positive exciton chirality; long arrows show the long axes of the two naphthalene chromophores.

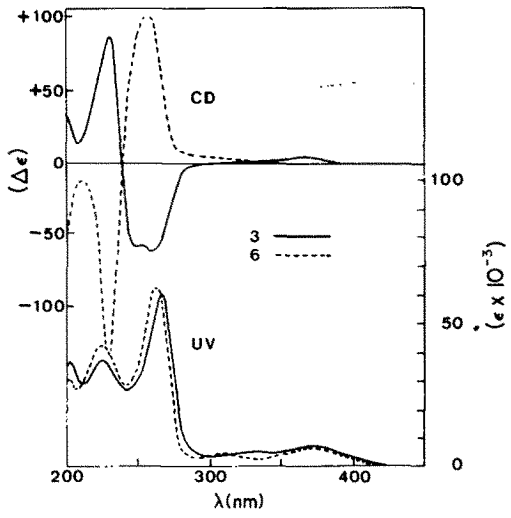



Fig. 3. CD and UV spectra of torosachrysone dimethyl ether monobenzoate (**3**) and germichrysone dimethyl ether monobenzoate (**6**), the CD curves of torosachrysone (**1**) and germichrysone (**4**) are much weaker and cannot be seen on this $\Delta\varepsilon$ scale

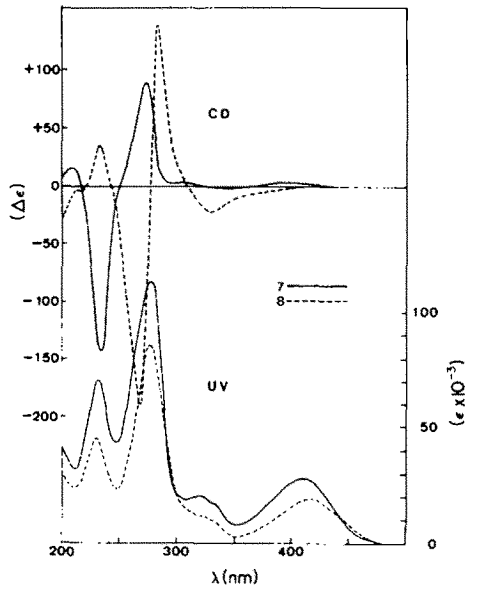



Fig. 4. CD and UV spectra of singueanol-I (**7**) and singueanol-II (**8**).

assigned to the 3-benzoate group on the bases of the W-type coupling between 2(equatorial)-H and 4(equatorial)-H, and the absence of such couplings between the 3-Me/2(axial)-H and 3-Me/4(axial)-H.[17]

The CD curve of the dimethyl ether monobenzoate (**3**) clearly exhibits very strong negative first (262 nm, $\Delta\varepsilon = -62.2$) and positive second (229 nm, $\Delta\varepsilon = +87.7$) Cotton effects due to a coupling between the $^1B_b$ transition (266 nm, $\varepsilon = 60{,}300$) of the naphthalene chromophore and the $^1L_a$ transition of the benzoate chromphore (Fig. 3); in contrast, no significant Cotton effects are observed in CD curve of torosachrysone (**1**) itself. The negative first and positive second Cotton effects are due to the negative exciton chirality, i.e. an anticlockwise twist between the two long axes of the naphthalene chromophore and the benzoate chromophore (Fig. 2A). This leads to the absolute configuration depicted in Fig. 2A, a *S*-configuration. The absolute configurations at 3 and 3′ in singueanol-I (**7**) and singueanol-II (**8**) are also presumably *S*. The CD curves (Fig. 4) of singueanol-I (**7**) and singueanol-II (**8**) exhibit strong positive first and negative second Cotton effects due to the couplings between $^1B_b$ transitions of the naphthalene chromophores, and this shows that the two long axes are twisted in a clockwise sense as depicted in Fig. 5.

The chiral exciton coupling was also employed to confirm the stereochemistry of germichrysone (**4**). Methylation and subsequent benzoylation gave the dimethyl ether monobenzoate (**6**). Spin–spin couplings between 3-H and 2-H, J = 5.8 Hz and J = 4.5 Hz, suggest the 3-H configuration to be equatorial and hence to be an axial configuration for the 3-benzoate (Fig. 2B). The CD curve (Fig. 3) of germichrysone dimethyl ether monobenzoate (**6**) exhibits very strong positive first (258 nm, $\Delta\varepsilon = +102.1$) and negative second (229 nm, $\Delta\varepsilon = -138.6$) Cotton effects due to a positive exciton chirality, i.e. a clockwise screwness between the two long axes of the naphthalene chromophore ($^1B_b$ transition: 262 nm, $\varepsilon = 63{,}500$) and the benzoate chromophore ($^1L_a$ transition, Fig. 2B); in contrast, no significant Cotton effects are observed in CD curve of germichrysone (**4**) itself. The R-configuration thus determined is in agreement with the results obtained by the Bijvoet method.[11]

## EXPERIMENTAL

All m.ps are uncorrected. PMR and CMR spectra were recorded on a JEOL FX-100 FT-NMR spectrometer in $CDCl_3$ unless otherwise stated, using TMS as an internal standard, the chemical shifts are expressed in $\delta$ values (ppm). The high resolution mass spectra were recorded on a JEOL-01SG-2 mass spectrometer. IR spectra were recorded on a Hitachi 260-10 IR spectrophotometer in KBr discs ($cm^{-1}$). UV spectra were recorded on a Hitachi 200-20 double beam spectrophotometer in MeOH; $\lambda_{max}$ are shown in nm ($\varepsilon$). CD curves were recorded on a JASCO J-20C automatic recording spectropolarimeter in MeOH; the maxima and minima are shown in nm and $\Delta\varepsilon$.

*Extraction and purification.* Pulverised *Cassia singueana* roots (1 kg) were extracted with $CHCl_3$ (3 × 3,000 ml) in a Waring blendor at 3,000 rpm for 1 min, and filtered. The $CHCl_3$ soln was evaporated *in vacuo* to dryness. The residue was chromatographed through a $SiO_2$ column and eluted with $CHCl_3$–MeOH (50:1) to obtain a tetrahydroanthracene

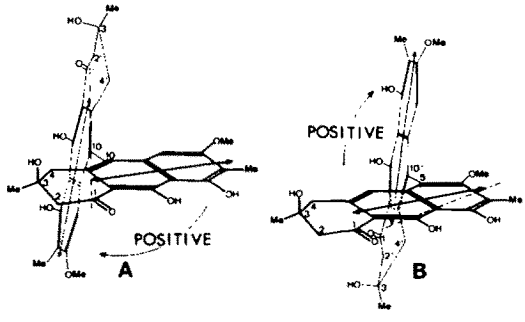



Fig. 5. Stereo views of the positive exciton chiralities of singueanol-I (**7**) shown in (A) and singueanol-II (**8**) shown in (B).

mixture. The mixture was then chromatographed through a LH-20 (Pharmacia) column and eluted with $CHCl_3$/MeOH (2:1). Singueanol-II (**8**, 50 mg), singueanol-I (**7**, 84 mg), torosachrysone (**1**, 134 mg), and germichrysone (**4**, 53 mg) were eluted successively and recrystallized from $CH_2Cl_2$/MeOH.

*Torosachrysone* (**1**), yellow needles; m.p. 183–186°; see Ref. 9 for MS, IR, UV, and PMR; CMR ($CDCl_3$/$CD_3OD$): 203.2 (s), 163.8 (s), 159.7 (s), 141.5 (s), 136.7 (s), 117.9 (d), 108.7 (s), 108.2 (s), 101.2 (d), 99.9 (d), 70.5 (s), 55.7 (q), 51.4 (t), 43.4 (t), 28.5 (q).

*Torosachrysone dimethyl ether monobenzoate* (**3**). Torosachrysone (**1**, 20 mg) was methylated with $CH_2N_2$ in ether at room temp (for 10 hr). The product was purified on $SiO_2$ tlc developing with $C_6H_6$/EtOAc (3:2) to give **2**, (12 mg); PMR: 7.20 (1H, s, 10-H), 6.54 (1H, d, J = 2.5 Hz, 5-H), 6.40 (1H, d, J = 2.5 Hz, 7-H), 3.91 (3H, s, -OMe), 3.87 (6H, s, OMe), 3.10 (2H, s, 4-H), 2.77 (2H, s, 2-H), 1.92 (1H, broad s, 3-OH), 1.38 (3H, s, 3-Me). The dimethyl ether (**2**, 12 mg) was treated with benzoyl chloride in pyridine at room temp (for 10 hr) and then water (50 ml) was added to decompose the excess reagent. The product was extracted with ether, which was washed with water and dried over $Na_2SO_4$. After removal of the ether, the product was purified on $SiO_2$ tlc developing with $CHCl_3$ to give **3** (13.4 mg) as pale yellow needles; m.p. 148–149° (MeOH/$CHCl_3$); $M^+$ 420.1580, $C_{25}H_{24}O_6$ requires 420.1571; IR: 2,910, 1,695, 1,670, 1,260, 700; UV: 225 (36,900), 266 (60,300), 320 (5,000), 332 (5,200), 366 (7,600); CD: 262 (−62.2), 229 (+87.7); PMR: 7.65–7.85 (2H, m), 7.10–7.50 (4H, m), 6.57 (1H, d, J = 2.5 Hz, 5-H), 6.42 (1H, d, J = 2.5 Hz, 7-H), 4.00 (1H, broad d, J = 17.0 Hz, $2_{eq}$-H), 3.93 (3H, s, -OMe), 3.88 (3H, s, -OMe), 3.87 (3H, s, -OMe), 3.27 (1H, d, J = 17.0 Hz, $4_{ax}$-H), 3.00 (1H, broad d, J = 17.0 Hz, $2_{eq}$-H), 2.88 (1H, d, J = 17.0 Hz, $2_{ax}$-H), 1.80 (3H, s, 3-Me).

*Germichrysone* (**4**), yellow needles; m.p. 190–193°; see Ref. 10 for MS, IR, UV, and PMR; CMR ($CDCl_3$/$CD_3OD$): 203.6 (s), 157.8 (s), 144.2 (s), 140.1 (s), 135.2 (s), 118.4 (d), 112.8 (d), 111.1 (s), 110.1 (s), 65.8 (d), 46.7 (t), 38.2 (t), 22.2 (q).

*Germichrysone dimethyl ether monobenzoate* (**6**). Germichrysone **4** (15.5 mg) was methylated and purified, as described for **1**, to yield 4.3 mg of **5**; PMR: 7.13 (1 H, broad s, 10-H), 6.97 (1 H, d, J = 2.0 Hz, 5-H or 7-H), 6.53 (1 H, d, J = 20 Hz, 5-H or 7-H), 4.48 (1 H, m, 3-H), 3.92 (3 H, s, -OMe), 3.86 (3 H, s, -OMe), 3.18 (2 H, m, 4-H), 2.83 (2 H, m, 2-H), 2.42 (3 H, s, 6-Me), 1.78 (1 H, broad s, 3-OH). The ether **5** (4.3 mg) was benzoylated and purified, as described for **2**, to give 1.5 mg of **6** as a pale yellow gummy substance, which was left *in vacuo* to crystallize as yellow needles; m.p. 114–117°; $M^+$ 390.1475, $C_{24}H_{22}O_5$ requires 390.1465; IR: 2,940, 1,720, 1,690, 1,280, 710; UV: 224 (42,700), 262 (63,500), 310 (5,200), 320 (3,900), 373 (7,700); CD: 258 (+102.1), 229 (−138.6); PMR: 7.80–7.93 (2 H, m), 7.25–7.60 (4 H, m), 7.16 (1 H, broad s, 5-H or 7-H), 6.65 (1 H, broad s, 5-H or 7-H), 5.66 (1 H, m, 3-H), 3.98 (3 H, s, -OMe), 3.94 (3 H, s, -OMe), 3.40 (2 H, m, 4-H), 3.05 (2 H, m, 2-H), 2.46 (3 H, s, 6-Me).

*Singueanol-I* (**7**), yellow needles; m.p. 119–122° (dec); $M^-$ 602.2153, $C_{34}H_{34}O_{10}$ requires 602.2149; IR: 3,380, 2,950, 1,625, 1,590; UV: 233 (70,100), 278 (114,000), 320 (21,800), 334 (17,100), 417 (27,900); CD: 273 (+89.4), 235 (−146.0); PMR: 16.67 (2H, s, 9,9′-OH), 10.36 (2H, s, 8,8′-OH), 5.98 (2H, s, 5,5′-H), 3.47 (6H, s, 6,6′-Me), 2.80 (4H, s, 2,2′-H), 2.51 (4H, s, 4,4′-H), 2.17 (6H, s, 7,7′-Me), 1.51 (2H, broad s, 3,3′-OH), 1.28 (6H, s, 3,3′-Me); CMR ($CDCl_3$/$CD_3OD$): 203.5 (s), 164.8 (s), 156.4 (s), 138.3 (s), 134.7 (s), 124.4 (s), 111.4 (s), 108.6 (s), 108.0 (s), 96.5 (d), 70.1 (s), 55.6 (q), 51.3 (t), 41.3 (t), 28.8 (q), 7.9 (q).

*Singueanol-II* (**8**), yellow needles; m.p. 154–158° (dec); $M^-$ 602.2160, $C_{34}H_{34}O_{10}$ requires 602.2149; IR: 3,380, 2,940, 1,625, 1,600; UV: 231 (47,900), 278 (86,700), 317 (14,200), 331 (11,000), 413 (21,400); CD: 285 (+140.6), 269 (−193.3); PMR: 16.56 (1H, s, 9 or 9′-H), 16.08 (1H, s, 9 or 9′-OH), 10.28 (2H, s, 8,8′-OH), 6.35 (1H, broad s, 10-H), 5.92 (1H, s, 5′-H), 3.47 (3H, s, 6 or 6′-OMe), 3.34 (3H, s, 6 or 6′-OMe), 2.75 (8H, m, 2,2′,4,4′-H), 2.31 (3H, s, 7-Me), 2.11 (3H, s, 7′-Me), 1.26 (6H, s, 3,3′-Me), 1.22 (2H, broad s, 3,3′-OH); CMR: 202.7 (s), 202.5 (s), 165.7 (s), 165.4 (s), 162.2 (s), 161.4 (s), 157.0 (s), 156.5 (s), 139.1 (s), 136.9 (s), 134.5 (s), 122.4 (s), 117.1 (s), 115.7 (d), 115.1 (s), 110.9 (s), 109.9 (s), 109.0 (s), 108.4 (s), 107.8 (s), 97.0 (d), 70.7 (s), 60.3 (q), 55.4 (q), 50.7 (t), 50.4 (t), 42.9 (t), 41.3 (t), 29.7 (q), 29.1 (q), 9.2 (q), 7.9 (q).

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