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Structural Identification of an FD&C Red No. 40 Contaminant

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ABSTRACT

NMR and mass spectral experiments have resulted in the determination of structure and assignment of carbon NMR signals of a manufacturing impurity of FD&C Red No. 40. Mass spectrometry showed its molecular weight to be 270, with a base peak of 228. A variety of two-dimensional NMR techniques demonstrated that this compound is a benzonaphthobicyclo-[2.2.2]octanone, which may arise from the Diels–Alder reaction of a naphthyl-benzyne species with 2-naphthol.

1 INTRODUCTION

FD&C Red No. 40 (**R40**) is an important certifiable azo dye approved for use in foods, drugs, and cosmetics. It is synthesized by coupling diazotized cresidine sulfonic acid with Schaeffer's salt (6-hydroxy-2-naphthalene sulfonic acid, sodium salt). An impurity (**1**) in commercial batches of the dye was found to originate as a contaminant of Schaeffer's salt. Structural identification of **1** was pursued because its mass spectrum was similar to those of several carcinogenic polynuclear aromatic hydrocarbons and because of the possible need for specifications limiting its presence in FD & C

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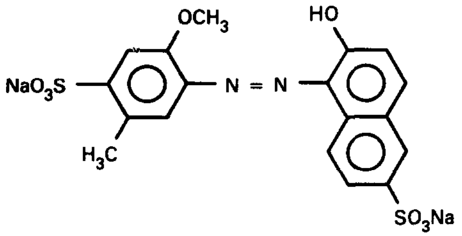
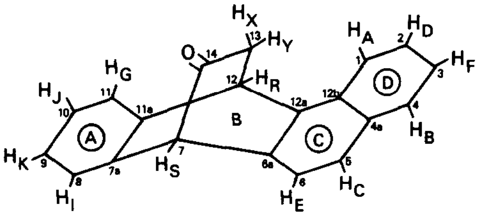


R40



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Red No. 40. NMR spectroscopy, in particular an indirectly bonded heteronuclear chemical-shift-correlation (FLOCK) experiment,[1] demonstrated that **1** is structurally dissimilar to both R40 and its immediate synthetic precursors and that it is a diarylbicyclo[2.2.2]octanone rather than a polynuclear aromatic hydrocarbon. The occurrence and levels of this contaminant in commercial lots of FD&C Red No. 40 were not determined because the contaminant did not present a known safety concern.

2 EXPERIMENTAL

2.1 Materials

Schaeffer's salt, an intermediate used as the coupling agent in the manufacture of FD&C Red No. 40, served as the starting material for the isolation of **1**. This intermediate was provided by Warner Jenkinson Co. (St Louis, MO 63106). Commercial HPLC-grade chloroform was used to extract the compound of interest from Schaeffer's salt. Commercial HPLC-grade acetonitrile and water were used for both the preparative and analytical HPLC separations.

2.2 Extraction of 1

Schaeffer's salt (151 g) was extracted with chloroform by using a Tecator Soxtec System H2T 1045 Extractor Unit (66 g) or Soxhlet extractor (85 g). In one experiment, Schaeffer's salt (33 g) was added to a cellulose thimble (33 × 80 mm, Fisher Scientific, Pittsburgh, PA). A plug of glass wool was placed on top of the Schaeffer's salt. The oven temperature of the Soxtec 1046 Service Unit was set at 180°C to heat the silicon oil-temperature control. HPLC-grade chloroform (70 ml) was then added to the unit, and the instrument was set to the boiling mode and allowed to reflux for 2·75 h. The instrument was set to the rinse mode, and the thimble was washed for 40 min.

The chloroform extract was cooled to room temperature and transferred to a 100-ml round-bottom flask. A rotary evaporator was used to remove the solvent at 30°C. A crude extract (0·14 g), containing **1** as a component, was obtained from the Soxtec extraction. A Soxhlet extractor, used for one extraction, gave similar results but with a longer extraction time. A total of 0·67 g of crude extract was obtained from multiple extractions.

2.3 Preparative HPLC of 1

Preparative HPLC was performed by using a Waters Delta Prep 3000 system operated at 50 ml/min with a Waters Delta Pak C-18 column (15 μm, 30 cm × 57 mm i.d.). A Linear Model 204 UVIS detector (254 and 288 nm) was employed with the detector cell set for a 0·5-mm pathlength. HPLC-grade water was used as solvent A for gradient chromatography. Solvent B was HPLC-grade acetonitrile.

The column was equilibrated with 2 liters of 20% water/80% acetonitrile at a flow rate of 50 ml/min. The residue containing **1** was dissolved in 120 ml of 30% water/70% acetonitrile and the solution was injected. A linear gradient, from 80% to 100% solvent B over 40 min, was used to achieve separations. Fractions were collected according to detector response. At the end of the run, a 20-min wash with 100% acetonitrile eluted remaining components from the column.

It was determined by analytical HPLC that the compound of interest was a component that eluted in the range of 15·7–17·4 min during the preparative HPLC separation. Fractions containing **1** were combined and rotary-evaporated. After solvent removal, 0·22 g of pure **1** remained.

2.4 Analytical HPLC of 1

Fractions collected during the preparative HPLC run were subjected to analytical HPLC to determine the elution profile of **1**. A Waters 600 E gradient liquid chromatograph operated at 1 ml/min and equipped with a Waters 490 E detector (254 and 288 nm) was used with a Nova-Pak C-18 column (4 μm, 100 × 8 mm i.d.) and a μBondpak C-18 Guard-Pak pre-column. Solvent A was HPLC-grade water and solvent B was HPLC-grade acetonitrile. The column was equilibrated for 15 min with 25% solvent B (75% solvent A). A linear gradient, from 25% to 100% solvent B over

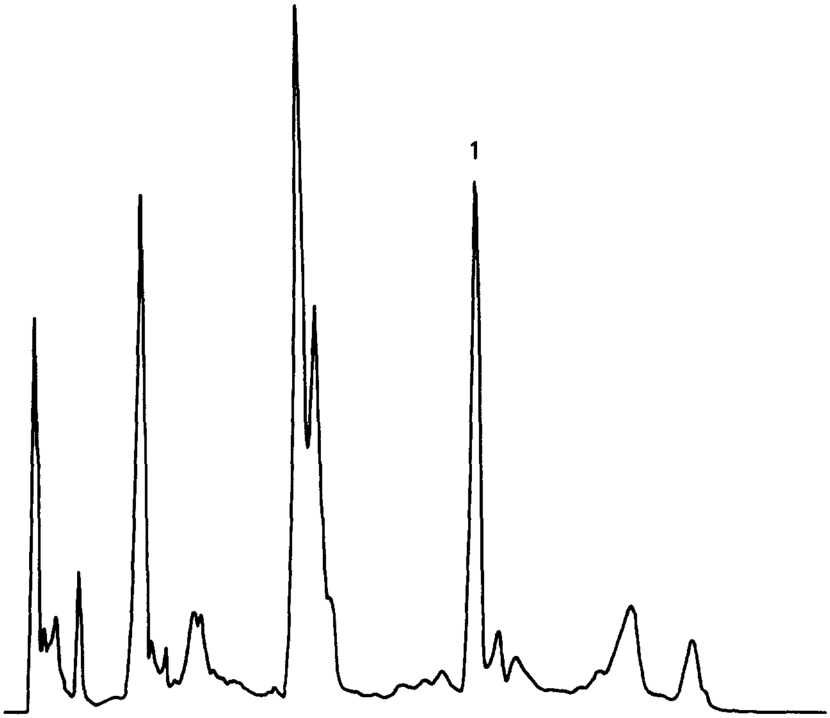


Fig. 1. High-performance liquid chromatogram of a commercial lot of FD&C Red No. 40.

20 min, was used to achieve separations. At the end of each run, a 15-min wash with 100% acetonitrile eluted remaining components from the column.

Analytical HPLC was performed for the combined extracts of Schaeffer's salt that served as the starting material for the preparative HPLC run. The resulting chromatogram (Fig. 1) shows that **1** elutes at 22·6 min when the analytical HPLC method described is used and that it represents *c.* 15% of the total absorbance at 254 nm. Multiple fractions were obtained that contained only a single component, corresponding to the retention time of **1** (Fig. 2). The fractions were combined and rotary-evaporated to dryness.

2.5 Mass-spectral analysis of 1

High-resolution mass-spectral experiments were conducted on a VG-MMZAB-2F mass spectrometer operated in the electron-ionization mode. Operating parameters were 70-eV electron energy, 200-μA emission current, and 200°C source temperature. High-resolution mass measurements were made by using the peak-matching method at 10 000 resolution and 5%

Fig. 2. High-performance liquid chromatogram of **1**.

valley. Perfluorokerosene was used as the reference standard. The test sample was introduced into the instrument via the solid probe. Accurate mass values obtained were *m*/*z* 270·104 38 ($C_{20}H_{14}O$, 0·08-mmu error) and 228·094 78 ($C_{18}H_{12}$, 0·8-mmu error).

Positive-ion chemical-ionization (CI) data were obtained on a VG-MMZAB-2F mass spectrometer at 1000 resolution. The operating parameters were 100-V electron energy, 0·5-mA emission current, and 180°C source temperature. Methane was used as the CI-reagent gas at a source pressure of 2×10^{-5} mbar. Mass-spectral data were acquired by using an INCOS 2300 data system scanning from *m*/*z* 460 to 60 in 3·0 s. The test sample was introduced via the solid probe.

2.6 Infra-red spectral analysis of 1

Infra-red spectra were obtained by using a Mattson Sirius 100 Fourier-transform spectrometer with a mercury–cadmium–telluride detector in the spectral range 4000 to 600 cm^{-1}. The test sample was analyzed in a KBr disk with a spectral resolution of 4 cm^{-1}. Characteristic group frequencies (cm^{-1}) were as follows: 3069 (vvw), 3048 (vw), 3024 (vvw), aromatic-ring CH stretching; 2972 (vw), methylene asymmetric stretching; 2938 (vvw), CH (tertiary) stretching; 2908 (vvw), methylene symmetric stretching; 1724 (vs), six-membered ring carbonyl stretching; 1624 (vvw), 1589 (vvw), 1516 (w), 1475 (w), 1458 (w), aromatic-ring C=C stretching; 1109 (wm), 1097 (wm), carbonyl C–C–C stretching; 809 (wm), two adjacent free hydrogens out-of-plane deformation in an aromatic ring; and 765 (wm), 744 (m), four adjacent free hydrogens out-of-plane deformation in an aromatic ring.

2.7 Proton and ^{13}C NMR analyses of 1

Proton and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, in $CDCl_3$ on a Varian Associates VXR-400 spectrometer

TABLE 1
^{1}H and ^{13}C Chemical Shifts of **1**[a]

Position	*C-13*	*^{n}H*	*H-1 (J, Hz)*
1	122·2	1	8·30 dd (7·7, 1·3)
2	126·4	1	7·62 dt (7·7, 1·3)
3	125·6	1	7·50 dt (7·6, 1·0)
4	128·8	1	7·89 dd (7·6, 1·0)
4a	132·8	0	
5	126·8	1	7·72 d (8·3)
6	123·7	1	7·54 d (8·3)
6a	134·7	0	
7	63·3	1	5·04
7a	137·6	0	
8	125·4	1	7·44 dd (7·4, 1·4)
9	126·5	1	7·19 dt (7·4, 1·4)
10	127·2	1	7·24 dt (7·4, 1·4)
11	123·9	1	7·49 dd (7·4, 1·4)
11a	142·3	0	
12	39·7	1	5·41 t (2·6)
12a	138·9	0	
12b	128·6	0	
13	37·8	2	2·37 dd (17·7, 2·6) 2·46 dd (17·7, 2·6)
14	205·3	0	

[a] In $CDCl_3$, referenced to TMS at 0·00 ppm.

(Table 1). The proton NMR spectrum was described by 16 384 data points (real part). Pulse widths of 30 μs were employed, which correspond to tip angles of 90° with 5-mm sample tubes. Spectral widths of 4000 Hz were used, corresponding to acquisition times of 4·1 s.

A proton-decoupled ^{13}C NMR spectrum, described by 32 768 data points (real part), was obtained with broad-band irradiation at 400 MHz. Pulse widths of 5 μs were employed, which correspond to tip angles of 35° with 5-mm sample tubes. Spectral widths of 25 kHz were used, corresponding to acquisition times of 1·3 s. Both proton and ^{13}C chemical shifts are reported relative to TMS.

An edited, proton-decoupled ^{13}C NMR spectrum (APT) was determined under conditions similar to those described above with the following changes. Pulse widths of 7 μs were employed, corresponding to tip angles of 45° with 5-mm sample tubes. Tau (D2) delays of 7 ms were used for full editing and observation of all resonances. Refocusing (D3) delays of 1 ms were employed, for reinversion of residual z-magnetization, together with 1-s repetition rates (D1).

A COSY NMR spectrum was recorded by using a spectral width of 1500 Hz in each domain and with 256 data points in the F2 dimension. A ^1H pulse width of 30 μs (90°) and a 1-s repetition rate were used to acquire 128 incremented proton NMR spectra of 16 scans each. Free-induction decays were processed as a 512 × 512 matrix with appropriate zero filling and pseudo-echo weighting.

A directly bonded, heteronuclear chemical-shift-correlation (HETCOR) NMR spectrum was obtained with spectral widths of 11 161 and 2668 Hz in the carbon and proton dimensions, respectively, and with 1024 data points in the ^{13}C dimension. Pulse widths of 13 μs (^{13}C, 90°) and 19·2 μs (^1H decoupler, 90°) and a 1-s repetition rate were employed to acquire 256 incremented ^{13}C NMR spectra of 16 scans each. Free-induction decays in both dimensions were processed as a 512 × 2048 matrix with appropriate zero filling and modified pseudo-echo weighting. A value of $^1J(CH) = 140$ Hz was employed for calculating the delays Δ_1 and Δ_2.

An indirectly bonded, heteronuclear chemical-shift-correlation (FLOCK) NMR spectrum was obtained with spectral widths of 17 272 and 2668 Hz in the carbon and proton dimensions, respectively, and with 2048 data points in the ^{13}C dimension. Pulse widths of 13 μs (^{13}C, 90°) and 19·2 μs (^1H decoupler, 90°) and a 1-s repetition rate were used to acquire 512 incremented ^{13}C NMR spectra of 96 scans each. Free-induction decays in both dimensions were processed as a 1024 × 2048 matrix with appropriate zero filling and modified pseudo-echo weighting. A value of $^nJ(CH) = 7{\cdot}5$ Hz was used for calculating the delays Δ_1 and Δ_2, and $^1J(CH) = 140$ Hz was used for τ in the BIRD pulses.

3 RESULTS AND DISCUSSION

A CI (positive-ion) mass spectrum of **1** displayed a molecular ion of $271(m+1)$, indicating a molecular weight of 270. High-resolution electron-impact mass spectra yielded a molecular ion of 270·1044, which is consistent with an empirical formula of $C_{20}H_{14}O$ and requires 14 units of unsaturation.

The infra-red spectrum of **1** showed a characteristic carbonyl-stretching vibration at 1724 cm^{-1}. This absorption indicated the presence of a ketone function that is either part of an acyclic compound or incorporated into a six-membered, or larger, ring system. The higher frequencies of the methylene stretching suggested that this group is attached to a carbonyl function.

COSY NMR spectra revealed the presence of an aliphatic 3-spin system ($H_RH_XH_Y$), an aromatic/olefinic 2-spin system (H_CH_E), two aromatic 4-spin systems ($H_AH_DH_FH_B$ and $H_GH_JH_KH_I$), and an isolated proton (H_S). ^{13}C NMR demonstrated the presence of ten sp^2-hybridized methine, six sp^2-hybridized quaternary, one carbonyl, one methylene, and two sp^3-hybridized methine carbons. Of particular interest were the considerably deshielded proton signals at 5·04 (H_S) and 5·41 ppm (H_R). A HETCOR NMR experiment established that these protons are attached to carbons whose chemical shifts are 63·3 and 39·7 ppm, respectively; H_S is, therefore, also aliphatic.

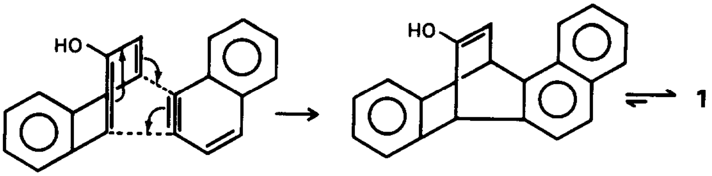
A carbon–hydrogen chemical-shift-correlation NMR experiment,[1] optimized for two- and three-bond C–H couplings, permitted the following connectivities to be established (Table 2): (i) the carbonyl function is located between H_S and H_XH_Y, (ii) H_S is situated between C-6a and C-7a and is three-bond-coupled to C-11a and C-12a, and (iii) H_R is positioned between C-11a and C-12a and is three-bond-coupled to C-6a and C-7a. From these connectivities, it can also be seen that C-6a is joined to C-12a and C-7a is attached to C-11a. Compound **1** is thus a diarylbicyclo[2.2.2]octanone.

The above heteronuclear chemical-shift-correlation experiment allowed additional three-bond C–H connectivities to be made (Table 2): (i) H_G to C-7a and H_I to C-11a, (ii) H_E to C-7 and C-12a, (iii) H_C to C-4 and C-12b, (iv) H_A to C-4a and C-12a, and (v) H_B to C-12b. Combination of this FLOCK-experiment information with that from the COSY experiment, which identified proton-spin-system members (e.g. ADFB), revealed that **1** is a benzonaphthobicyclo[2.2.2]octanone with the following proton-group assignments: contiguous GJKI placed in the A-ring, adjacent CE located in the C-ring, and contiguous ADFB positioned in the D-ring. Moreover, protons A and G are situated adjacent to H_R, and protons I and E flank H_S. The considerably downfield chemical shifts of the bridgehead protons (R

TABLE 2
FLOCK Experiment: Indirect C–H Connectivities[a]

Position	*^1H Connectivities*	*Position*	*^1H Connectivities*
1	B, D, and F	8	J, K, and S
2	A and B	9	G
3	A, B, and D	10	I and K
4	A, D, and C	11	J, K, R, and S
4a	A, F, C, and E	11a	I, J, R, S, X, and Y
5	B and E	12	G, X, and Y
6	C, R, and S	12a	A, E, R, S, X, and Y
6a	C, E, R, and S	12b	A, B, D, C, and R
7	E and I	13	R and S
7a	G, K, R, and S	14	R, S, X, and Y

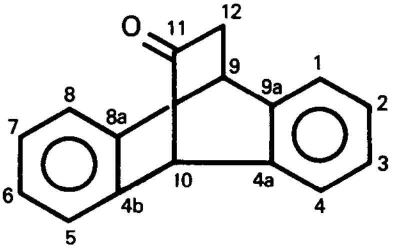
[a] In $CDCl_3$.



Scheme 1.

and S) probably reflect their doubly benzylic nature, since they exist simultaneously in the planes of two aromatic rings.[2] Finally, the stereochemistry of H_X and H_Y can be determined from the relative intensities of the correlation signals in the FLOCK experiment. H_X displays a strong correlation to C-12a and a weak correlation to C-11a, whereas H_Y exhibits just the opposite behavior. These correlation intensities are indicative of the magnitude of the respective three-bond couplings, which are themselves a function of the dihedral angles between these four nuclei.[3] A Dreiding model of this bicyclo[2.2.2]octanone system shows that these dihedral angles are either 60° or 180°. The more intense correlations, which are associated with 180°-dihedral-angle relationships,[3] occur between H_X and C-12a and between H_Y and C-11a. H_X must, therefore, be *cis* to the A-ring and H_Y must be *cis* to the C-ring.

Compound **1** is then seen to possess a fused phenyl ring (A) at the 7a,11a-positions and an *a*-fused naphthyl ring (C,D) at the 6a,12a-positions. It is, as previously mentioned, structurally quite dissimilar to both R40 and the starting materials from which R40 is synthesized. Although the source of this contaminant is not at present known, it could conceivably arise from a Diels–Alder reaction during the sulfonation process used to produce Schaeffer's salt from 2-naphthol. Hot, concentrated sulfuric acid, which is employed in this vigorous reaction, is a strong dehydrating agent.[4] It may be able to effect the dehydration of unreacted 2-naphthol to form a naphthyl–benzyne ('naphthyne') species. Benzynes are highly reactive dienophiles[5] that, *inter alia*, undergo Diels–Alder reactions with dienes, even those as unreactive as benzene.[6] If the above naphthyne intermediate is produced, it could participate in a Diels–Alder reaction with additional unreacted 2-naphthol to yield **1** (Scheme 1). The bisphenyl analog (**2**) of **1** is synthesized



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by the Diels–Alder reaction of anthracene and either (i) methyl acrylate followed by oxidation[7] or (ii) 2-acetoxyacrylonitrile followed by hydrolysis.[8]

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