Val-L, column no. 314734, Chrompak, Inc.).

Blank tests were carried out in the same way with exactly the same samples of amino acids or esters that were used as precursors for the pentapeptide synthesis. This included H-Gly-OH, H-Phe-OH, H-Tyr-OH, and H-Leu-OH (2 mg of each). Results for the three chiral amino acids as percent of D form were 0.69 (Phe), 1.01 (Tyr), and 0.60 (Leu). Analogous results for the blanks were 0.83 (Phe), 0.89 (Tyr), and 0.63 (Leu). The figures given are the averages for three independent determinations.

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# New Mycotoxins from *Fusarium sambucinum*

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Isolation, characterization, and bioassay results of three new trichothecene mycotoxins, 3-ketoapotrichothecene (**6**), FS-3 (**9**), and FS-4 (**11**), as well as a new bisaboline, 4,5,10,11-tetrahydroxybisaboline (**14**), from *Fusarium sambucinum* are presented. 3,15-Diacetoxyscirpenol (3,15-DAS) (**1**), FS-1 (**10**), and 3$\alpha$- and 3$\beta$-hydroxyapotrichothecenes (**7**, **8**) are reported for the first time from *F. sambucinum*. The previously reported 4,15-diacetoxyscirpenol (4,15-DAS) (**2**) (anguidine), 4-monoacetoxyscirpenol (4-MAS) (**3**), 15-MAS (**4**), 3,4,15-triacetoxyscirpenol (TAS) (**5**), neosolaniol, sambucoin, and scirpenetriol were also found. The solid-state structure of DAS (**2**) (anguidine) obtained from single-crystal X-ray analysis is reported.

The trichothecene mycotoxins found in *Fusarium*-damaged grains[1,2] are responsible for alimentary toxic aleukia (ATA), skin inflammation, vomiting, and death in humans and farm animals. Our recent studies of *Fusarium sporotrichioides*, which produces large amounts of T-2 toxin, revealed a number of new trichothecenes.[3-7] *Fusarium sambucinum*, which produces DAS (4,15-diacetoxyscripenol), also known as anguidine, another potent toxin, in significant quantity, as well sambucinol, sambucoin,[8] and sambucinic acid,[9] was cultured and followed by chromatographic workup procedures previously described.[7] We now report the structure elucidation, spectral data, and preliminary bioassay results of a new 4,5,10,11-tetrahydroxybisaboline; three unusual modified trichothecenes 3-ketoapotrichothecene, FS-3, FS-4; as well as the isolation of three known metabolites 3,15-DAS, FS-1, 3$\alpha$- and 3$\beta$-hydroxyapotrichothecenes not reported previously from *F. sambucinum*. Sambucoin, scirpenetriol, neosolaniol, 4-MAS, 15-MAS, and 3,4,15-triacetoxyscirpenol have been found in *F. sambucinum* by others.[8,10-12] To our knowledge, this is the first report of a bisaboline produced by a fungus.

A large-scale workup of the culture filtrate used a modified method of Burmeister.[13] Approximately 400 jars were harvested in batches of 100–200 jars over a period of 1 year. The corn grits were extracted with $CHCl_3$–$Me_2CO$ (85:15) (400 mL/jar) by blending at high speed until homogenized. The fungal–solvent mixture was allowed to stand overnight and suction filtered. The solid residue was reextracted with $Me_2CO$, suction filtered, autoclaved, and discarded. The $Me_2CO$ extract was combined with the $CHCl_3$–$Me_2CO$ (85:15) extract and concentrated under vacuum. The dark-red oil (~0.5 L/200 jars) was jubjected to a hexane drip to remove the nonpolar constituents. This was achieved by dripping the oil into a stirring solution of hexane–$Me_2CO$ (85:15) (ca. 50 mL oil/2 L solution) and allowing to stand for 24 h. The solvent was decanted and concentrated under vacuum.

The majority of DAS (**2**) was removed from the oil by crystallization from $Me_2CO$–hexane. Approximately 100 g of **2** was obtained from the 400 jars (0.25%). Multiple runs of 2-g aliquots of the remaining red oil were flash chromatographed.[14] A gradient solvent system was used employing toluene; toluene–$Me_2CO$ (4:1), (2:1), (1:1); $Me_2CO$; and 1:1 $Me_2CO$–MeOH. Each of the six resultant fractions were further separated by flash chromatography. Oil from each fraction was applied to a 4 × 45 cm flash

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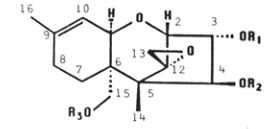
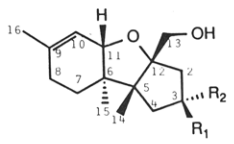
column containing flash grade Si gel. Eighty 20-mL fractions were collected and concentrated under vacuum. Fraction 1 contained only fatty material arising from corn grits and was not subjected to further chromatography.

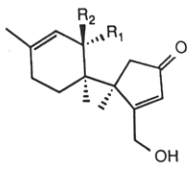
Two grams of fraction 2 were applied to a 4 × 45 cm flash column and eluted with 4:1 toluene–$Me_2CO$. This fraction contained fatty material, DAS (**2**), 3,15-DAS (**1**), 3,4,15-triacetoxyscirpenol (**5**), neosolaniol, sambucoin, 4-MAS (**3**), 15-MAS (**4**), and FS-3 (**9**). The 15-MAS, 4-MAS, and neosolaniol were purified by normal-phase HPLC using 4:1 toluene–$Me_2CO$, with yields of 2 mg (50 ppb), 2 mg (50 ppb), and 1.7 mg (40 ppb), respectively. Sambucoin, FS-3 (**9**), 3,15-DAS (**1**), and TAS were purified by normal-phase HPLC using 4:1 toluene–$Me_2CO$, followed by preparative TLC with 3:1 toluene–$Me_2CO$. Approximate yields were sambucoin (1.7 mg, 40 ppb), FS-3 (3 mg, 70 ppb), TAS (1.5 mg, 35 ppb) and 3,15-DAS (1.7 mg, 40 ppb).



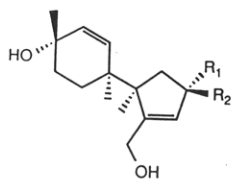
| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| **1** | Ac | H | Ac |
| **2** | H | Ac | Ac |
| **3** | H | Ac | H |
| **4** | H | H | Ac |
| **5** | Ac | Ac | Ac |



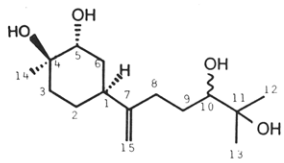
**6** $R_1 = R_2 = O$
**7** $R_1$ = H, $R_2$ = OH
**8** $R_1$ = OH, $R_2$ = H



**9** $R_1 = R_2 = O$
**10** $R_1$ = OH, $R_2$ = H



**11** $R_1 = R_2 = O$
**12** $R_1$ = H, $R_2$ = OH
**13** $R_1$ = OH, $R_2$ = H



**14**

Fractions 3 and 4 proved an abundant source of new modified trichothecenes. Fraction 3 was eluted with 2:1 toluene–acetone. Further cleanup of **6** was done by preparative normal-phase thin-layer chromatography, yielding 4 mg (0.1 ppm). Fraction 4 was eluted with 1:1 toluene–$Me_2CO$, in which FS-1 (**10**), bisabolene (**14**), FS-4 (**11**), 3$\alpha$- and 3$\beta$-hydroxyapotrichothecenes (**7**, **8**), and scirpenetriol were obtained. Scirpenetriol, FS-1, and FS-4 (**11**) were purified by reverse-phase HPLC using 5:1 $H_2O$–ACN yielding 10 mg (0.25 ppm), 15 mg (0.37 ppm), and 900 $\mu$g (20 ppb), respectively. Bisabolene (**14**) and apotrichothecenes **7** and **8** were purified by RP-TLC using 7:3 MeOH–$H_2O$, resulting in 5 mg (0.12 ppm), 1.2 mg (50 ppb), and 900 $\mu$g (20 ppb), respectively.

Fractions 5 and 6 were analyzed using C-18 flash chromatography. Both fractions were eluted individually by a gradient solvent system consisting of $H_2O$–MeOH (3:1), (2:1), (1:1); MeOH. No trichothecenes were found in these fractions.

3,15-Diacetoxyscirpenol (3,15-DAS) (**1**), previously reported from *F. roseum* and tentatively characterized by mass spectrometry,[15] has an IR spectrum indicative of

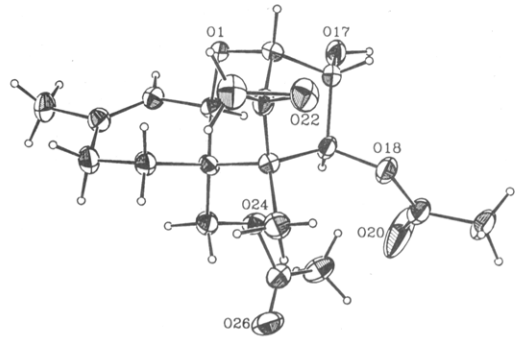



**Figure 1.** Computer-generated perspective drawing (ORTEP) of independent molecule A of DAS (**2**).

hydroxyl, carbonyl, and ether functionalities. The UV spectrum ($\lambda_{max}$ 199, $\epsilon_{max}$ 8950, ACN) supported an ene $\pi$–$\pi^*$ transition. The $^1H$ NMR data (Table I) shows the presence of a vinylic ($\delta$ 1.73, 3 H, b s), two acetate ($\eta$ 2.05, 2.20, both s), and a tertiary methyl ($\delta$ 0.87, 3 H, s), as well as the 12,13-epoxide methylene protons ($\delta$ 2.79, 3.05, both d, $J$ = 4.0 Hz). COSY[16] allowed the gross structure of **1** to be depicted as shown, *without* placement of the acetates, and made proton assignments possible. The acetates were assigned by comparing the chemical shifts of 3-H, 4-H, and 15-H's of **1** and DAS **2** (4,15-DAS). The only significant difference is the 3-H in **2** is upfield ($\delta$ 4.13) and the 4-H is downfield ($\delta$ 5.13) compared to 3-H ($\delta$ 4.85) and 4-H ($\delta$ 4.31) in **1**. The 15-H's and the 2-H/3-H, 3-H/4-H coupling constants of **1** and **2** are practically identical (Table I). The 4-H was shown to be coupled to OH, which clearly indicates that the acetates of **1** are on C-3 and C-15. The $^{13}C$ NMR spectral data (Table I) supported the structure of **1** as depicted.

Reports of X-ray crystal structures of other trichothecenes[17–19] led us to attempt to obtain crystals of 4,15-DAS (**2**) suitable for X-ray analysis. Recrystallization of 4,15-DAS (**2**) (anguidine) from cyclohexane/$Me_2CO$ gave colorless crystals, which were used in single-crystal X-ray analysis. ORTEP drawings of one of the two independent molecules are shown in Figure 1. The two independent molecules are not significantly different. Positional parameters of **2** are given in Table II.

The known trichothecenes 4-MAS (**3**), 15-MAS (**4**), 3,4,15-triacetoxyscirpenol (**5**) (Table I), scirpenetriol, sambucoin,[8] and neosolaniol were identified by comparison of spectral data. FS-1 (**10**) was identical in all respects to an authentic sample obtained from *F. sporotrichioides*.[5] The 3$\alpha$- and 3$\beta$-hydroxyapotrichothecenes (**7**, **8**) have been reported previously from another fungal source.[20–24] The $^1H$ NMR spectral data of **7** and **8** were identical with those previously reported for 3$\alpha$- and 3$\beta$-hydroxyapotrichothecenes,[23–25] although the stereochemistry at C-11

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**Table I. NMR Data on 1-5 in $CDCl_3$ ($^1H$ NMR at 300 MHz, $^{13}C$ NMR at 75.5 MHz)**

| | 1 | | 2 | | | | |
|---|---|---|---|---|---|---|---|
| atom | $^1H$ | $^{13}C$ | $^1H$ | $^{13}C$ | 3, $^1H$ | 4, $^1H$ | 5, $^1H$ |
| 2 | 3.81 (4.9) | 77.6 | 3.64 (4.9) | 77.4 d | 3.68 (4.8) | 3.59 (4.2) | 3.86 (4.8) |
| 3 | 4.85 (2.9, 4.9) | 83.5 | 4.13 (3.0, 4.9) | 78.6 d | 4.26 (3.1, 4.8) | 4.20 m | 5.20 (3.3, 4.8) |
| 4 | 4.31 (2.9) | 79.1 | 5.13 (3.0) | 83.8 d | 5.53 (3.1) | 4.20 m | 5.75 (3.3) |
| 5 | | 48.9 | | 48.4 s | | | |
| 6 | | 43.6 | | 43.5 s | | | |
| 7 | | 20.9 | | 20.7 t | | | |
| | 1.7–2.1 | | 1.7–2.1 | | 1.7–2.2 | 1.5–2.1 | 1.6–2.1 |
| 8 | | 28.0 | | 27.5 t | | | |
| 9 | | 141.0 | | 140.0 s | | | |
| 10 | 5.47, b d (5.5) | 118.2 | 5.49 (5.3) | 118.1 d | 5.58 b d (5.0) | 5.46 b d (5.0) | 5.49 b d (5.7) |
| 11 | 3.75, b d (5.5) | 68.1 | 4.06 (5.3) | 67.5 d | 4.20 b d (5.0) | 3.92 b d (5.0) | 3.99 b d (5.7) |
| 12 | | 64.0 | | 64.0 s | | | |
| 13 | 2.79 | 46.8 | 2.74 | 46.7 t | 2.78 | 2.72 | 2.81 |
| | 3.05 (4.0) | | 3.01 (4.0) | | 3.06 (4.0) | 3.00 (3.9) | 3.09 |
| 14 | 0.87 s | 6.7 | 0.77 s | 6.7 q | 0.86 s | 0.77 s | 0.78 s |
| 15 | 3.90 | 63.5 | 3.94 | 63.1 t | 3.64 | 3.83 | 4.05 |
| | 4.13 (12.3) | | 4.12 (12.5) | | 3.69 (12) | 4.19 (12.2) | 4.26 (12.3) |
| 16 | 1.73 b s | 23.2 | 1.68 b s | 22.8 q | 1.74 b s | 1.69 b s | 1.73 b s |
| $COCH_3$ | 2.05 | 21.0 | 2.00 | 20.6 q | 2.17 | 2.03 | 2.07 |
| | 2.20 | 21.2 | 2.16 | 20.6 q | | | 2.11 |
| $COCH_3$ | | no[a] | | 171.6 s | | | |
| | | no[a] | | 170.2 s | | | |

[a] No = not observed due to small sample size.

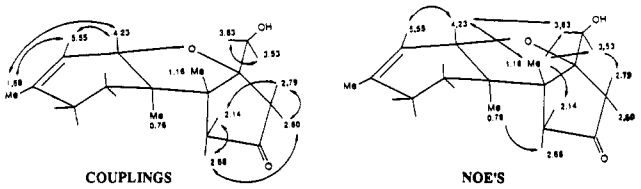


**Figure 2.** Couplings from COSY and NOE's from NOESY and DNOES on 3-ketoapotrichothecene (**6**).

was initially shown incorrectly[19-21] and is correct as depicted.[23-25]

3-Ketoapotrichothecene (**6**), $C_{15}H_{22}O_3$, has bands in its IR spectrum appropriate for hydroxyl and carbonyl groups. The UV spectrum ($\lambda_{max}$ 197, $\epsilon_{max}$ 8600, ACN) was assigned as an ene $\pi$–$\pi^*$ transition. From inspection of the $^1H$ NMR spectrum (300 MHz, $CDCl_3$) one vinyl ($\delta$ 1.68) and two methyl singlets ($\delta$ 0.76, 1.16), two isolated *W*-coupled methylenes ($\delta$ 2.60, 2.79, $J_{AB}$ = 20.0 Hz; $\delta$ 2.14, 2.68, $J_{AB}$ = 19.2 Hz), a methylene next to and coupled to hydroxyl ($\delta$ 3.53, 3.83, $J$ = 3, 11.3 Hz), and an allylic methine next to oxygen ($\delta$ 4.23, m) coupled to a vinyl proton ($\delta$ 5.55, m) were observed. The $^1H$ NMR data of **7** and **8** indicated they were closely related to **6**. COSY, NOESY,[26] and DNOES (difference nuclear Overhauser effect spectroscopy) experiments were useful in determination of structure and facilitated proton assignments (Table III). The couplings (COSY) and NOE's are summarized in Figure 2, confirming the overall structure of **6** as depicted.[27] $^{13}C$ NMR spectral data are in accord with **6** (Table III). Apotrichothecene **6** was previously isolated from *F. sporotrichioides* (MC-72083) in our laboratory,[28] but limited sample size precluded detailed spectroscopic analyses.

FS-3 (**9**), $C_{15}H_{20}O_3$, has an IR spectrum consistent with hydroxyl and conjugated carbonyl functionalities. The UV spectrum ($\lambda_{max}$ 229 nm, ACN) further substantiated the presence of conjugated carbonyl functionality. The $^1H$ NMR spectrum of **9** closely resembled that of FS-1 (**10**)[5] as seen in Table III, with two significant differences. The H-11 seen at $\delta$ 4.27 in **10** is clearly missing in **9**, and the H-10 of **10** at $\delta$ 5.12 is shifted downfield to $\delta$ 5.75 in **9**. These data are consistent with replacing a hydroxyl at C-11 in **10** with a carbonyl group in **9**. COSY and NOESY experiments allowed proton assignments to be made in **9** and are shown in Table III. Comparison of the $^{13}C$ NMR data (Table III) of **9** and **10** also indicated they were closely related. Key differences were again found in the A ring, with the C-11 methine at $\delta$ 71.2 in **10** absent in **9**, replaced by a new quaternary peak at $\delta$ 202.1 in **9** appropriate for a conjugated ketone. The above data are in accord with the structure of **9** as shown.

FS-4 (**11**) has an IR spectrum indicative of the presence of hydroxyl and conjugated carbonyl groups. The $^1H$ NMR spectral data of **11** were assigned using COSY and NOESY (Table III). The H-2, H-4, and H-12 protons of **11** (B ring) are similar to those found in **9** and **10**, indicating the presence of the same cyclopentenone ring system in **11**. However, the A ring of **11** contained an isolated ene ($\delta$ 5.52, d, $J$ = 10 Hz, coupled to $\delta$ 5.61), which we had observed in FS-2 (**12**) ($\delta$ 5.58, d, H-10, $J$ = 10.2 Hz coupled to $\delta$ 5.67, H-11),[7] trichodiol,[7] trichotriol,[7] and 3-epi-FS-2 (**13**) isolated from *F. sporotrichioides* earlier.[28] This suggests that the A rings are the same in all compounds. The $^{13}C$ NMR data, although not complete due to small sample size, support the structure assignment, with good agreement in chemical shifts between in A ring carbons in **9**, **10**, and **11**. The ring B carbons of **11** (Table III) also match those of **12**, trichodiol, and trichotriol (C-7, $\delta$ 27.8, 27.7, 27.8; C-8, 35.0, 35.3, 34.8; C-10, 135.6, 133.8, 136.0; C-11, 133.2, 133.5, 132.3, respectively)[7] quite well, given solvent and B ring structural differences. The above data are consistent with the structure as depicted. No molecular ion was observed

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**Table II. Fractional Atomic Coordinates and Equivalent Isotropic Thermal Parameters[a]**

| | x | y | z | B, Å² |
|---|---|---|---|---|
| O1A | 0.7803 (4) | −0.053 | 0.7205 (2) | 2.88 (8) |
| O17A | 0.8295 (5) | 0.1978 (4) | 0.7598 (2) | 4.00 (9) |
| O18A | 0.8366 (5) | 0.2976 (4) | 0.6102 (2) | 3.80 (9) |
| O20A | 0.6199 (6) | 0.4202 (6) | 0.6045 (4) | 12.7 (2) |
| O22A | 1.0343 (5) | 0.0353 (5) | 0.6014 (2) | 4.8 (1) |
| O24A | 0.3911 (4) | 0.1707 (4) | 0.6258 (2) | 3.43 (8) |
| O26A | 0.2638 (6) | 0.2479 (5) | 0.5333 (2) | 5.5 (1) |
| C2A | 0.9044 (6) | 0.0202 (6) | 0.6981 (3) | 3.1 (1) |
| C3A | 0.8811 (7) | 0.1629 (6) | 0.7027 (3) | 3.2 (1) |
| C4A | 0.7587 (6) | 0.1976 (5) | 0.6424 (3) | 3.0 (1) |
| C5A | 0.7320 (6) | 0.0746 (5) | 0.6018 (3) | 2.8 (1) |
| C6A | 0.5870 (6) | −0.0054 (5) | 0.6227 (2) | 2.5 (1) |
| C7A | 0.5838 (7) | −0.1439 (6) | 0.5970 (3) | 3.2 (1) |
| C8A | 0.4539 (7) | −0.2263 (6) | 0.6199 (3) | 4.1 (1) |
| C9A | 0.4346 (6) | −0.2046 (6) | 0.6875 (3) | 3.4 (1) |
| C10A | 0.5050 (6) | −0.1075 (5) | 0.7206 (3) | 3.0 (1) |
| C11A | 0.6145 (6) | −0.0131 (5) | 0.6959 (2) | 2.7 (1) |
| C12A | 0.8895 (6) | 0.0039 (6) | 0.6273 (3) | 3.3 (1) |
| C13A | 0.9706 (7) | −0.0942 (7) | 0.5956 (3) | 4.9 (2) |
| C14A | 0.7072 (8) | 0.1000 (7) | 0.5299 (3) | 4.3 (1) |
| C15A | 0.4196 (6) | 0.0503 (6) | 0.5959 (3) | 3.6 (1) |
| C16A | 0.3312 (8) | −0.2984 (7) | 0.7156 (3) | 4.9 (2) |
| C19A | 0.7556 (7) | 0.4053 (6) | 0.5965 (3) | 5.1 (2) |
| C21A | 0.8568 (9) | 0.5046 (7) | 0.5724 (4) | 6.7 (2) |
| C25A | 0.3071 (6) | 0.2591 (6) | 0.5894 (3) | 3.6 (1) |
| C27A | 0.2741 (8) | 0.3702 (7) | 0.6275 (3) | 5.0 (2) |
| O1B | 0.9240 (4) | 0.4443 (4) | 0.7740 (2) | 2.96 (8) |
| O17B | 0.9009 (5) | 0.6969 (4) | 0.7284 (2) | 4.05 (9) |
| O18B | 0.8295 (4) | 0.8040 (4) | 0.8688 (2) | 3.32 (8) |
| O20B | 1.0438 (6) | 0.9306 (5) | 0.8694 (4) | 10.2 (2) |
| O22B | 0.5977 (5) | 0.5557 (4) | 0.8585 (2) | 4.9 (1) |
| O24B | 1.2627 (4) | 0.6574 (4) | 0.9062 (2) | 3.47 (8) |
| O26B | 1.4197 (7) | 0.6776 (6) | 0.9999 (3) | 10.2 (2) |
| C2B | 0.7865 (7) | 0.5222 (6) | 0.7822 (3) | 3.3 (1) |
| C3B | 0.8213 (6) | 0.6648 (5) | 0.7795 (3) | 3.1 (1) |
| C4B | 0.9153 (6) | 0.6975 (5) | 0.8452 (2) | 2.8 (1) |
| C5B | 0.9066 (6) | 0.5775 (5) | 0.8881 (3) | 3.0 (1) |
| C6B | 1.0570 (6) | 0.4881 (5) | 0.8837 (3) | 2.7 (1) |
| C7B | 1.0291 (7) | 0.3518 (6) | 0.9074 (3) | 3.0 (1) |
| C8B | 1.1672 (7) | 0.2594 (6) | 0.9002 (3) | 3.6 (1) |
| C9B | 1.2319 (7) | 0.2740 (5) | 0.8395 (3) | 3.5 (1) |
| C10B | 1.1943 (6) | 0.3727 (5) | 0.8018 (3) | 3.1 (1) |
| C11B | 1.0792 (6) | 0.4775 (5) | 0.8130 (2) | 2.5 (1) |
| C12B | 0.7573 (6) | 0.5128 (6) | 0.8502 (3) | 3.2 (1) |
| C13B | 0.6432 (7) | 0.4243 (7) | 0.8725 (4) | 5.2 (2) |
| C14B | 0.8871 (8) | 0.6087 (6) | 0.9559 (3) | 4.0 (1) |
| C15B | 1.2148 (7) | 0.5349 (6) | 0.9250 (3) | 3.5 (1) |
| C16B | 1.3382 (8) | 0.1675 (6) | 0.8238 (4) | 5.3 (2) |
| C19B | 0.9042 (7) | 0.9149 (6) | 0.8751 (3) | 4.2 (1) |
| C21B | 0.7940 (7) | 1.0200 (6) | 0.8855 (3) | 4.0 (1) |
| C25B | 1.3703 (8) | 0.7193 (7) | 0.9488 (3) | 5.0 (2) |
| C27B | 1.4248 (9) | 0.8396 (7) | 0.9222 (4) | 6.2 (2) |

[a] Anisotropically refined atoms are given in the form of the isotropic equivalent displacement parameter defined as:

$$(4/3)[aB(1,1) + b^2B(2,2) + c^2B(3,3) + ab(\cos\gamma)B(1,2) + ac(\cos\beta)B(1,3) + bc(\cos\alpha)B(2,3)]$$

in EIMS and CIMS of **11**. This was probably due to facile cleavage between C-5 and C-6, leading to stable charged species. Exact mass measurements supported this, giving *two* $m/z$ 125 peaks for $C_8H_{13}O$ *and* $C_7H_9O_2$, which also accounts for the expected molecular formula of $C_{15}H_{22}O_3$.

4,5,10,11-Tetrahydroxybisaboline (**14**), $C_{15}H_{26}O_6$, has a band in its IR spectrum suggestive of a hydroxyl functionality. The UV spectrum indicated one ene $\pi$–$\pi^*$ transition ($\lambda_{max}$ 195, $\epsilon_{max}$ = 5000), with no other chromophore present. Inspection of the $^1H$ NMR spectrum showed the presence of three methyl singlets with chemical shifts consistent with $\beta$-oxygen substitution ($\delta$ 1.17, 1.22, 1.27), two methines bearing oxygen ($\delta$ 3.38, dd, $J$ = 1.7, 10.4 Hz; $\delta$ 3.64, b t, $J$ = 3.0 Hz) and a 1,1′-disubstituted ene ($\delta$ 4.79, b m; $\delta$ 4.84, b s). COSY and NOESY exper-

**Table III. NMR Spectra Data on 6–11 in $CDCl_3$ ($^1H$ NMR at 300 MHz and $^{13}C$ NMR at 75 MHz)**

| | **6** | | **7**[23] | | **8**[24] | | **9** | | **10**[5] | | **11** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| atom | $^1H$ | $^{13}C$ | $^1H$ | $^{13}C$[a] | $^1H$ | $^{13}C$ | $^1H$ | $^{13}C$ | $^1H$ | $^{13}C$ | $^1H$ | $^{13}C$ |
| 2 | 2.60 (2.2, 20.0) | 49.6 t | 1.74 m | 44.4 t | 1.38 m | 47.0 t | 6.29 (t, 1.5) | 129.6 d | 6.32 (t, 1.5) | 129.7 d | 6.27 (t, 1.5) | 128.1 |
| | 2.79 (1.4, 20.0) | | 2.62 (1.7, 6.1, 12.3) | | 2.45 (1.3, 5.8, 6.4) | | | | | | | |
| 3 | | 215.5 s | 4.30 b m | 72.8 d | 4.51 b m | 74.2 d | | 207 s | | 207.6 s | | 205 |
| 4 | 2.14 (1.4, 19.2) | 49.6 t | 1.64 m | 43.5 t | 2.10 | 45.7 t | 2.32 (18.8) | 50.6 t | 2.09 (18.0) | 50.1 t | 2.15 (19.0) | 49 |
| | 2.68 (2.2, 19.2) | | 2.15 (10.3, 13.0) | | 2.23 | | 2.60 (18.8) | | 2.95 (18.0) | | 2.73 (19.0) | |
| 5 | | 51.3 s | | 52.5 s | | no[b] | | 51.3 s | | 52.8 s | | no[b] |
| 6 | | 46.2 s | | 44.7 s | | no[b] | | 47.1 s | | 41.9 s | | no[b] |
| 7 | 1.48 m | 28.8 t | 1.40 (2, 4, 5.8, 13.0) | 27.8 t | 1.36 m | 27.5 t | 1.73 (2, 5, 12) | 30.9 t | 1.83 m | 29.8 t | 1.40 m | 28.2 |
| | 1.63 m | | 1.58 m | | 1.57 m | | 1.87 (2, 5, 12) | | 1.99 m | | 1.70 m | |
| 8 | 2.05 m | 26.8 t | 1.62 b m | 29.3 t | 1.99 m | 29.3 t | 2.20 (2, 5, 19) | 28.3 t | 1.48–1.6 m | 27.3 | 1.70 m | 32.5 |
| | 2.15 m | | 2.02 m | | | | 1.63 (2, 5, 19) | | | | 1.85 m | |
| 9 | | 135.7 s | | 135.5 s | | no[b] | | 160.1 s | | 136.5 s | | no[b] |
| 10 | 5.55 m | 210.4 d | 5.55 b splet | 121.9 d | 5.50 | 122.5 d | 5.75 b s | 126.4 d | 5.12 b s | 124.9 d | 5.52 (10) | 133.6 |
| 11 | 4.23 m | 80.1 d | 4.18 b splet | 81.3 d | 4.11 | 81.3 d | | 202.5 s | 4.27 b s | 71.2 d | 5.61 (1, 5, 10) | 130.9 |
| 12 | | 90.8 s | | 92.5 s | | no[b] | | 186.3 s | | 186.9 | | 183 |
| 13 | 3.53 (8.0,[c] 11.3) | 65.0 t | 3.18 (b t, 11.1) | 63.3 t | 3.56 (11.4) | 65.5 t | 4.49 (1, 5, 17) | 61.6 t | 4.41 (16.5) | 61.1 t | 4.69 (17) | 62 |
| | 3.83 (11.3) | | 3.77 (dd, 8, 11.1) | | 3.78 (11.4) | | 4.56 (1, 5, 17) | | 4.60 (16.5) | | 4.35 (17) | |
| 14 | 1.16 s | 19.2 q | 1.02 s | 19.2 q | 1.07 s | 20 q | 1.50 s | 23.8 q | 1.34 s | 21.1 q | 1.29 s | 19.2 |
| 15 | 0.76 s | 15.3 q | 0.95 s | 17.9 q | 0.52 s | 16.5 | 1.17 s | 18.2 q | 0.87 s | 12.9 q | 0.98 s | 18.0 |
| 16 | 1.68 s | 22.6 q | 1.50 (q, 1.4) | 22.6 q | 1.63 b s | 22.5 q | 1.93 s | 29.0 q | 1.68 s | 22.5 q | 1.25 s | 29.0 |

[a] In benzene-$d_6$. [b] No = not observed. [c] OH coupling.

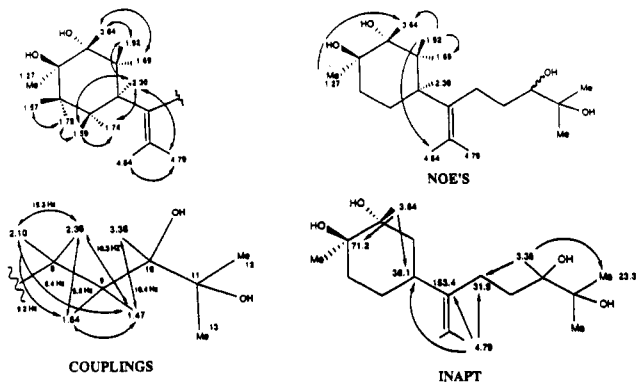


**Figure 3.** $^1H$ NMR assignments and relative stereochemistry obtained from COSY and NOESY on 4,5,10,11-tetrahydroxybisaboline (**14**) with the long-range heternuclear correlations found using INAPT ($CDCl_3$).

iments allowed proton assignments to be made on the cyclohexane ring and side chain of **14** (Figure 3). The $^{13}C$ NMR data suggested that **14** was not related to any trichothecenes found previously. Using DEPT ($Me_2CO$-$d_6$), three methyls ($\delta$ 25.1, 25.9, 28.1), two methines bearing oxygen ($\delta$ 74.1, 78.7), two quaternary carbons bearing oxygen ($\delta$ 70.8, 72.8) five $sp^3$ methylenes ($\delta$ 27.7, 31.0, 31.9, 34.6, 35.8), an aliphatic methine ($\delta$ 31.7), and a 1,1'-disubstituted ene ($\delta$ 107.2, t; 156.1, s) were observed. To complete the structure, a series of long-range 1D heteronuclear INAPT[5,33] experiments were performed in $CDCl_3$ (due to peak overlap in $Me_2CO$-$d_6$), and are summarized in Figure 3. From the INAPT data, the structure of **14**, a novel bisaboline was determined. The relative stererochemistry was determined using the NOESY experiment and is as depicted. Efforts are under way to establish the complete stereochemistry of **14**. This is the first report of a bisaboline from any fungal source (to our knowledge) and is interesting because a bisaboline is on the biosynthetic pathway leading to trichodiene, a precurser to the trichothecenes.[34] The absolute stereochemistry of **1**, **6**, **9**, and **11** is assumed to be the same as all previously isolated trichothecenes.[17]

## Experimental Section

**Instrumentation.** All ultraviolet spectra was obtained on a Perkin-Elmer 576 ST spectrophotometer. Infrared spectra were obtained on a Fourier transform Nicolet 20 DXB. Samples were cast as a film on a NaCl plate. Mass spectra of the samples were obtained on a Kratos MS-25 mass spectrometer equipped with a DS-55 data system. The instrument was operated in several different modes: low resolution (1/66) electron impact (EI) at 70 eV and 1 s/decade scanning speed; medium resolution (1/6500) EI at 70 eV and 3 s/decade for measurement of exact masses. Perfluorokerosene was used for calibration during EIMS runs. All NMR experiments were performed on a Nicolet NT-300 WB spectrometer equipped with 5-mm $^1H$ and $^{13}C$ single-frequency probes operating at 300.06 and 75.45 MHz, respectively. All $^1H$ NMR chemical shifts were referenced to internal tetramethylsilane (TMS) (0.00 ppm), and all $^{13}C$ NMR chemical shifts were referenced against the deuterated solvent used ($CDCl_3$ = 77.0 ppm).

**Culture Conditions.** *F. sambucinum*, kindly identified by Professor Paul E. Nelson of Pennsylvania State University, was grown on yeast malt agar plates for 14 days at 25 °C. Sterilized $H_2O$ was added to the agar plates, and the conidia were scraped into a larger volume of sterilized distilled $H_2O$ (100 mL/agar plate). One hundred to two hundred one-quart Ball canning jars containing 100 g of Quaker Oats white corn grits were autoclaved for 30 min. Aliquots (2 mL) of the mycelium–$H_2O$ mixture were shaken and the lids loosened to allow for respiration. After 24 h of incubation at 10 °C in darkness, the jars were shaken again to insure complete dispersal of the mycelium. The jars were incubated for a total of 21 days at 10 °C in darkness.

**Isolation.** All solvents used for extraction and florisil chromatography were ACS grade purchased from Fisher. All solvents used for flash chromatography, HPLC, and TLC were glass-distilled solvents purchased from Burdick & Jackson. The florisil was Fisher 60–100 mesh and packed by pouring the florisil into a 5 × 20 cm gravity column ($C_6H_6$–hexane, 2:1) to depth of 8 cm. A 2-cm layer of Fisher anhydrous $Na_2SO_4$ was added on top of the florisil. Flash chromatography used EMR reagent 40-40-63 $\mu$m Kieselgel 60 silica gel. Normal-phase TLC plates were silica gel HLF uniplates, 250 m thick, purchased from Analtech. Prep TLC entailed applying 5–10 mg of material to a 10 × 20 cm silica gel Analtech HLF uniplate, 250 $\mu$m thick. After development, compounds were identified by their quenching behavior at 254 nm or by spray visualization using a chromogenic reagent. The bands of interest were scraped and sonicated with 10 mL of $Me_2CO$ for 1 min and allowed to stand for 2 h with occasional stirring. The sample was filtered through a Rainin 0.45 $\mu$m nylon-66 filter, concentrated under vacuum, and prepared for NMR analysis using standard procedures. Prep RPTLC plates were Whatman KC18F with 200 $\mu$m thickness and used the same procedure as described above for prep TLC. Prep HPLC was conducted on a Perkin-Elmer series 3B chromatograph using a linear gradient solvent system.

**3,15-Diacetoxyscirpenol (1)**: colorless glass; $^1H$ NMR (Table I); $^{13}C$ NMR (Table I); IR $\nu_{max}$ (film) 3403 (OH), 1731 (C=O) $cm^{-1}$; UV $\lambda_{max}$ (ACN) 199 nm ($\epsilon_{max}$ 8200); EIMS 307 (5, $M^+$ – OAc), 205 (68), 124 (100), 105 (97), 81 (55); exact mass for $C_{17}H_{23}O_5$ ($M^+$ – OAc) calcd 307.174, found 307.154.

**3-Ketoapotrichothecene (6)**: colorless glass; $^1H$ NMR (Table III); $^{13}C$ NMR (Table III); IR $\nu_{max}$ (film) 3420 (OH), 1737 (C=O) $cm^{-1}$; UV $\lambda_{max}$ (ACN) 197 nm ($\epsilon_{max}$ 8600); EIMS 250 (15, $M^+$), 235 (38), 124 (45), 107 (50), 43 (100); exact mass for $C_{15}H_{22}O_3$ ($M^+$) calcd 250.156, found 250.158.

**3$\alpha$-Hydroxyapotrichothecene (7)**: colorless glass; $^1H$ NMR (Table III); $^{13}C$ NMR (Table III); IR $\nu_{max}$ (film) 3400 (OH), $cm^{-1}$; UV $\lambda_{max}$ (ACN) 196 nm ($\epsilon_{max}$ 7200); EIMS 252 (28, $M^+$), 237 (17), 140 (100), 124 (51); exact mass for $C_{15}H_{24}O_3$ ($M^+$) calcd 252.186, found 252.173.

**3$\beta$-Hydroxyapotrichothecene (8)**: colorless glass; $^1H$ NMR (Table III); $^{13}C$ NMR (Table III); IR $\nu_{max}$ (film) 3420 (OH), 1048 (C–O–C) $cm^{-1}$; UV $\lambda_{max}$ (ACN) 197 nm ($\epsilon_{max}$ 7800); EIMS 252 (15, $M^+$), 237 (15), 124 (38), 107 (92), 83 (100); exact mass for $C_{15}H_{24}O_3$ ($M^+$) calcd 252.186, found 252.181.

**FS-3 (9)**: colorless glass; $^1H$ NMR (Table III); $^{13}C$ NMR (Table III); IR $\nu_{max}$ (film) 3470 (OH), 1675 (C=O) $cm^{-1}$; UV $\lambda_{max}$ (ACN) 229 nm ($\epsilon_{max}$ 13000); EIMS 248 (1, $M^+$), 181 (14), 131 (31), 124 (26), 69 (100); exact mass for $C_{15}H_{20}O_3$ ($M^+$) calcd 248.154, found 248.141.

**FS-4 (11)**: colorless glass; $^1H$ NMR (Table III); $^{13}C$ NMR (Table III); IR $\nu_{max}$ (film) 3450 (OH), 1690 (C=O) $cm^{-1}$; UV $\lambda_{max}$ (ACN) 198 nm ($\epsilon_{max}$ 10400); EIMS 235 (4, $M^+$ – Me), 125 (86), 108 (100, 81 (83); exact mass for $C_8H_{13}O$ ($M^+$ – $C_7H_9O_2$) calcd 125.097, found 125.096, $C_7H_9O_2$ ($M^+$ – $C_8H_{13}O$) calcd 125.060, found 125.059, $C_8H_{11}$ ($C_8H_{13}O$ – $H_2O$) calcd 107.086, found 107.086.

**4,5,10,11-Tetrahydroxybisaboline (14)**: colorless glass; $^1H$ NMR ($CDCl_3$) $\delta$ 1.17 (3 H, s, H-12), 1.22 (3 H, s, H-13), 1.27 (3 H, s H-14), 1.47 (1 H, M, H-9a), 1.57 (1 H, m, H-3a), 1.59 (1 H, m, 2a), 1.64 (1 H, m, H-9b), 1.69 (1 H, m, H-6a), 1.74 (1 H, m, H-2b), 1.78 (1 H, m, H-3b), 1.92 (1 H, m, H-6b), 2.10 (1 H, m, H-8a), 2.30 (1 H, m, H-1), 2.36 (1 H, m, H-8b), 3.38 (1 H, dd, $J$ = 1.7, 10.4 Hz, H-10), 3.64 (1 H, b t, $J$ = 3.0 Hz, H-5), 4.79 (1 H, b m, H-15a), 4.84 (1 H, m, H-15b); $^{13}C$ NMR ($Me_2CO$-$d_6$) $\delta$ 25.1 (q, C-12), 25.9 (q, C-13), 27.7 (t, C-2), 28.1 (q, C-14), 31.0 (t, C-6), (t, C-8), 34.6 (t, C-3), 35.8 (t, C-9), 37.1 (t, C-1), 70.8 (s, C-4), 72.8 (s, C-11), 74.1 (d, C-10), 78.7 (d, C-5), 107.2 (t, C-15), 156.1 (s, C-7); IR $\nu_{max}$ (film) 3376 (OH), 1370, 1158, 1124, 1048, 1050 (C–O–C, C–O–H) $cm^{-1}$; UV $\lambda_{max}$ (ACN) 195 nm ($\epsilon_{max}$ 5000); EIMS 254 (1, $M^+$ – $H_2O$), 239 (2), 236 (3), 218, (2), 125 (21), 108 (64), 81 (89), 43 (100); exact mass for $C_{15}H_{26}O_3$ ($M^+$ – $H_2O$) calcd 254.188, found 254.190.

(33) Bax, A.; Ferretti, J. A.; Nashed, N.; Jerina, D. M. *J. Org. Chem.* **1985**, *50*, 3029.

(34) Cane, D. E.; Ha, H.-J. *J. Am. Chem. Soc.* **1988**, *110*, 6865 and references therein.

**Single-Crystal X-ray Analysis of 4,15-DAS (2).** Crystal data for 4,15-diacetoxyscirpenol (**2**), $C_{19}H_{26}O_7$: clear colorless rod, 0.2 × 0.3 × 0.4 mm, monoclinic, space group $P2_1$, $a$ = 8.273 (3) Å, $b$ = 10.536 (3) Å, $c$ = 21.363 (5) Å, $\beta$ = 99.5 (1)°, $V$ = 1837 (2) Å$^3$, and $\rho_{calc}$ = 1.325 g/cm$^3$ for $Z$ = 4. Diffraction data: Enraf-Nonius CAD4 automated $\kappa$-axis diffractometer, graphite-monochromated Mo radiation ($\lambda$(K$\alpha$) = 0.710 73 Å), range 2.0 < 2$\theta$ < 45.0°, 2796 reflections (2487 unique, $R_i$ = 0.029, 2065 observed, $I > 2.0\sigma(F)$); corrected for anomalous dispersion, Lorentz and polarization effects, but absorption ignored ($\mu$ = 0.945 cm$^{-1}$). Solution: direct methods (SHELXS-86)[29] and difference Fourier syntheses. Refinement: anisotropic thermal coefficients for non-H atoms, hydroxyl H atoms located and refined, all other H atoms placed at calculated positions, all H atoms with fixed isotropic thermal parameters.[30] Final: no significant features in the final difference Fourier map (range −0.30 < e/Å$^3$ < +0.32); agreement factors, $R$ = 0.41, $R_w$ = 0.050, and $S$ = 1.019.

**Bioassay.** The specific-pathogen-free (SPF) fertile chicken eggs, used for the chick embryo bioassay were purchased from Larson Lab-Vac Eggs, Inc., P.O. Box 474, Gowrie, IA 50543. The incubators used were the Imperial II produced by Lab-Line Instruments, Inc., and a Model 3212-10 produced by National Appliance Company. The temperature was maintained at 37 °C, and the relative humidity was kept at ~60%. Embryos were received within 24 h of laying, incubated for 5 days, and candled for viability. Typically a batch of 150 eggs would yield 120 acceptable eggs. Before dosing, the air cells were circled with a pencil and swabbed with 200-proof EtOH (Midwest Solvents Company of Illinois). An 18-gauge needle, sterilized in 200-proof EtOH, was used to puncture the shell above the encircled air cell. The toxins were dissolved in 200-proof EtOH, serial diluted, and 10 $\mu$L injected into the air cell with a microsyringe. The hole was then sealed Scotch tape. 10 $\mu$L of 200-proof EtOH was injected into the control eggs. In four days the eggs were candled and the number of dead embryos were determined for each dose level. The new trichothecenes **1** and **6** were bioassayed for toxicity using the chick embryo inoculations methods.[31,32] Preliminary results suggest that **6** is much less toxic than DAS (**2**), which has an LD$^{50}$ value of 45 ng/egg.[31] However, **1** appears to be close to **2** in embryotoxicity.

**Acknowledgment.** We are grateful to Professor Paul E. Nelson of Pennsylvania State University for kindly identifying *F. sambucinum* and Charles H. Condra for technical assistance. We are also grateful for partial financial support from the National Science Foundation for support of the NMR (PCM-8115599) and MS (PCM-8117116) Facilities, the University of Missouri Institutional Biomedical Research Support Grant (RR07053) from the NIH (M.S.T.), and the USDA Animal Health Research Formula Fund (G.E.R.).

**Supplementary Material Available:** Table of general displacement parameter expressions (1 page). Ordering information is given on any current masthead page.

# Configurations of the Isomeric 7-Oxiranylbicyclo[4.2.0]octan-7-ols

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The four racemic diastereoisomers of the title compound **1**, prepared by epoxidation of the exo and endo vinyl precursors **4** and **5**, were assigned tentative configurations in an earlier study.[1] The corresponding bromohydrins (**6b,c**) from the [1$\alpha$,6$\alpha$,7$\beta$,7($S$*)]-(±)- and [1$\alpha$,6$\alpha$,7$\alpha$,7($S$*)]-(±)-isomers **1b** and **1c** were converted to cyclic carbonate derivatives **7** and **8**, the structures of which were established by X-ray analysis. This confirms the earlier assignments.

In an earlier report[1] we noted that the four racemic diastereoisomers of **1**, the title compound, each behaved in a different fashion when treated with catalytic boron trifluoride etherate under mild conditions. Three of the isomers underwent regio- and stereoselective rearrangements to ring-expanded hydroxymethyl-substituted ketones (**2a**, **2b**, and **3a**), and the fourth was recovered unchanged. Structural assignments for these hydroxymethyl ketone products were based on their $\beta$-elimination to characteristic enones and stereoselective conversion to corresponding cis or trans diols.

The isomeric epoxy cyclobutanols **1** were easily assigned as isomeric pairs, related to the exo and endo vinyl alcohols **4** and **5** from which they were prepared. Further identification within each pair proved impossible by spectroscopic methods, and we made tentative configurational assignments based on the stereoselectivity of the observed rearrangements.[2] These are shown in Scheme I; the configuration of the unreactive isomer **1b** being fixed by process of elimination.

On reaction with magnesium bromide, each of the epoxy cyclobutanol isomers (**1a–d**) gave a unique crystalline bromohydrin (**6a–d**), which reverted to the parent epoxide on mild base treatment (Scheme II). Clearly, an X-ray structure analysis of one bromohydrin from each pair (**6a** or **6b** and **6c** or **6d**) would establish unquivocally the configurations of all the epoxide isomers; however, all our efforts to obtain crystals suitable for this purpose were unsuccessful. Finally, assuming that cyclic ester derivatives of the bromohydrins might give better results, we studied their reactions with oxalyl chloride and were gratified to obtain good yields of useable products (**7** and **8** from **6b** and **6c**, respectively). Although we expected cyclic oxalates from this reaction, the $^{13}$C NMR spectra of **7** and **8** displayed single carbonyl signals at ca. $\delta$ 153 ppm, characteristic of cyclic carbonates.[3] X-ray diffraction analysis of these two isomers not only confirmed their cyclic carbonate structure but also established the relative configuration at C-9 (the side chain chiral center), as shown in Figure 1. Since this center remains fixed throughout all the conversions originating from **1** (and **6**), **1b** is thereby

(1) Hwang, C.-S.; Reusch, W. *Heterocycles* **1987**, *25*, 589.

(2) If these rearrangements proceed via an antiperiplanar orientation of the oxirane CH–O bond and the migrating ring residue, both molecular model (Dreiding) inspection and molecular mechanics calculations point to the assignments given in Scheme I.

(3) Levy, G. C.; Lichter, R. L.; Nelson, G. L. *Carbon-13 Nuclear Magnetic Resonance Spectroscopy*, 2nd ed.; Wiley: New York, 1980; p 156.

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