Stereochemistry of Nidurufin: Synthesis of 6,8-Dideoxynidurufin and 6,8-Dideoxyepinidurufin

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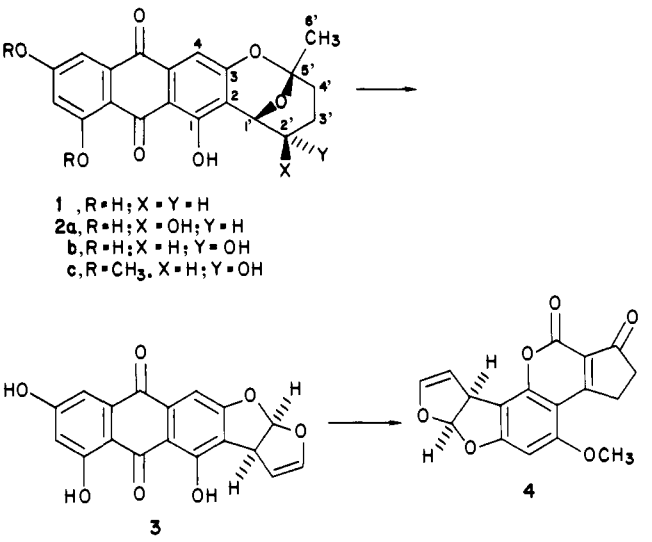
The extreme toxicity of the aflatoxins, a group of metabolites produced by certain strains of the mold *Aspergillus*, has generated tremendous interest in the biosynthesis of these compounds.[1] Averufin (**1**)[2] and versicolorin A (**3**)[3] have been shown to be pivotal intermediates in this biosynthesis and to be linked by successive rearrangement reactions which convert the C-6 linear side chain of averufin (**1**) to the branched C-4 dihydrobisfuran present in versicolorin A (**3**) and in aflatoxin B_1 (**4**) (Scheme I).[4] A C-2′ hydroxy derivative of averufin, nidurufin, has been isolated as a minor metabolite of *Aspergillus nidulans*[5] and has recently been suggested as a possible intermediate in the biosynthetic transformation of averufin (**1**) to versicolorin A (**3**).[6]

A novel alkylanthraquinone side-chain rearrangement, discovered in our laboratories,[6b] led us to consider the stereochemical aspects of nidurifin's role in the biosynthetic scheme. We reasoned that *exo*-2′-hydroxyaverufin (**2a**), if phosphorylated by an *Aspergillus* enzyme, would readily undergo the branching rearrangement sequence shown in Scheme II. This process involves the internal displacement of the C-2′ phosphorylated hydroxyl group by a correctly positioned C-2 aryl π-orbital as indicated by the electron arrows. Examination of molecular models shows that the *endo*-2′-hydroxyaverufin epimer (**2b**) is stereochemically incapable of undergoing an analogous AR_13 displacement reaction.

The C-2′ hydroxyl group of nidurufin has been assigned the endo configuration as in **2b** primarily on the basis of 100-MHz ^1H NMR spectral data,[5] but this assignment is not unambiguous since the unknown 2′-epinidurufin was not available for comparison. In view of the importance of the stereochemistry of the 2′-OH group for biosynthetic consideration, further evidence bearing on this point seemed highly desirable. In this regard, we now report a synthesis of the very close model compounds 6,8-dideoxynidurufin (**11**) and its C-2′ OH epimer (**10**) (Scheme III).

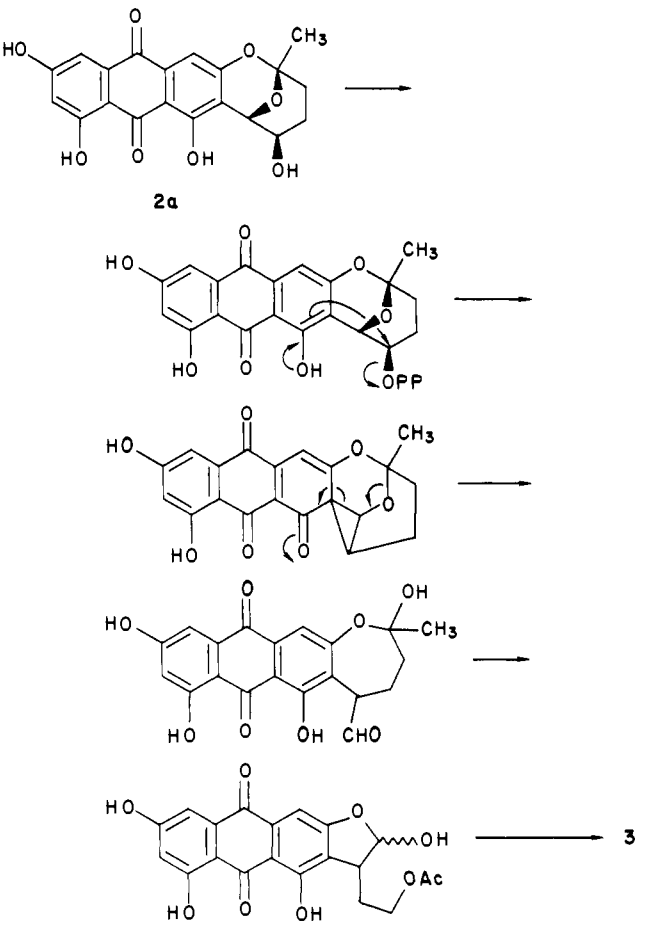
1,3-Dihydroxyanthraquinone (**5**)[7] was selectively protected at the non-hydrogen bonded 3-OH as a methoxymethyl ether and was then allylated at the 1-OH position. This allyl ether was subjected to reductive Claisen rearrangement conditions[8] placing a 2-propenyl side chain in the 2-position of the anthraquinone, and the 1-OH was then also protected as a methoxymethyl (MOM) ether to afford intermediate **6**. The 2-allyl side chain was smoothly converted to the transposed 3-chloro-1-propenyl compound using the "one-pot procedure" of Raucher[9] (kinetic addition of PhSeX followed by oxidative elimination of PhSe with 30% H_2O_2). A three-carbon chain homologation of this allylic chloride was accomplished using a Lewis acid catalyzed alkylation[10] with acetone enol silyl ether[11] to afford the trans-unsaturated ketone **7**. The *trans*-alkene system of **7** was converted cleanly to the *cis*-diol **8** by using catalytic OsO_4–$NaClO_3$[12] and also to

Scheme I



1, R = H; X = Y = H
2a, R = H; X = OH; Y = H
b, R = H; X = H; Y = OH
c, R = CH_3, X = H; Y = OH

Scheme II



(1) Steyn, P. S.; Vleggaar, R.; Wessels, R. L. In "The Biosynthesis of Mycotoxins"; Steyn, P. S., Ed.; Academic Press: New York, 1980; pp 105–155.

(2) Isolation: Holker, J. S. E.; Kagal, S. A.; Mulheirn, L. J.; White, P. M. *J. Chem. Soc., Chem. Commun.* **1966**, *24*, 911–913. Synthesis: Townsend, C. A.; Davis, S. G.; Christensen, S. B.; Link, J. C.; Lewis, C. P. *J. Am. Chem. Soc.* **1981**, *103*, 6885–6888.

(3) Hamasaki, T.; Hatsuda, Y.; Terashima, N.; Renbutsu, M. *J. Agric. Biol. Chem.* **1967**, *31*, 11–17.

(4) Asao, T.; Buchi, G.; Abdel-Kader, M. M.; Chang, S. B.; Wick, E. L.; Wogan, G. N. *J. Am. Chem. Soc.* **1965**, *87*, 882–886.

(5) Aucamp, P. J.; Holzapfel, C. W. *J. S. Afr. Chem. Inst.* **1970**, *23*, 40–56.

(6) (a) Sankawa, U.; Shimada, H.; Kobayashi, T.; Ebizuka, Y.; Yamamoto, Y.; Nuguchi, H.; Seto, H. *Heterocycles* **1982**, *19*, 1053–1057. (b) Ahmed, Z.; Cava, M. P. *J. Am. Chem. Soc.* **1983**, *105*, 682–684. (c) Kingston, D. G. I.; Chen, P. N.; Vercellotti, J. R. *Phytochemistry* **1976**, *15*, 1037–1039. (d) Townsend, C. A.; Christensen, S. B. *Tetrahedron* **1983**, *39*, 3575.

(7) Castonguay, A.; Brassard, P. *Can. J. Chem.* **1977**, *55*, 1324.

(8) Rutledge, P.; et al. *Tetrahedron Lett.* **1982**, 4407.

(9) (a) Miyano, M. *J. Org. Chem.* **1981**, *46*, 1846–1853. (b) Liotta, D.; Zima *Tetrahedron Lett.* **1978**, 4977. (c) Raucher, S. *Tetrahedron Lett.* **1977**, 3909.

(10) Reetz, M. T.; Huttenhain, S.; Walz, P.; Lowe, U. *Tetrahedron Lett.* **1979**, 4971–4974.

(11) House, H. O.; Czuba, L. J.; Gall, M.; Olmstead, H. D. *J. Org. Chem.* **1969**, *34*, 2324–2336.

(12) Wiesner, K.; Chan, K. K.; Demerson, C. *Tetrahedron Lett.* **1965**, 2893–2897.

(13) All compounds gave satisfactory elemental analysis and spectroscopic data.

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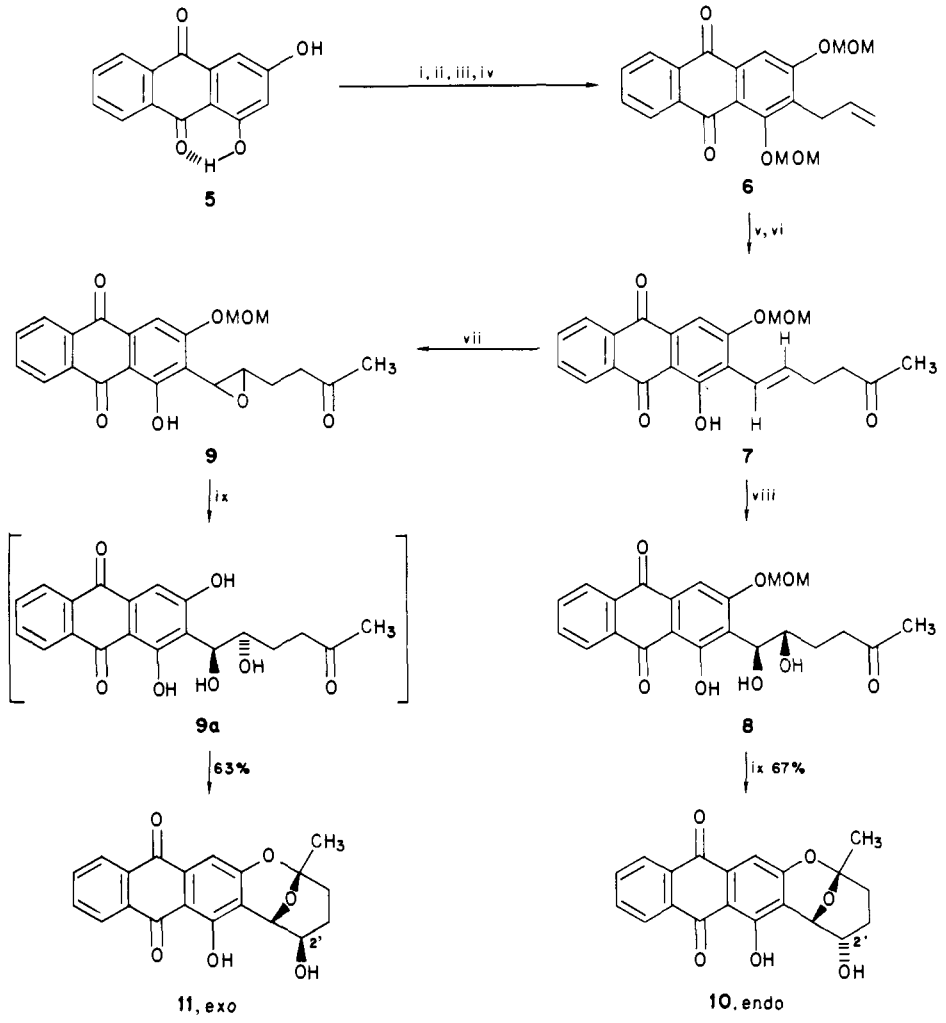
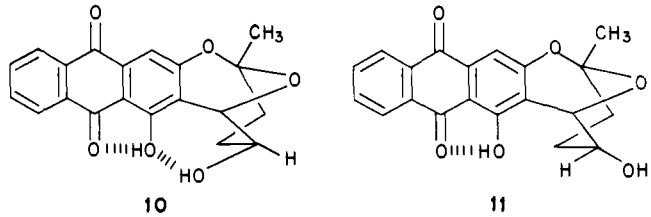
Scheme III[a]



[a] (i) $ClCH_2OCH_3$, *t*-BuOK, THF, room temperature (94%); (ii) allyl bromide, K_2CO_3, DMF, 70 °C (86%); (iii) $Na_2S_2O_4$, $NaHCO_3$, DMF-H_2O (1:1), 90 °C (89%); (iv) $ClCH_2OCH_3$, *t*-BuOK, THF, room temperature (92%); (v) (a) PhSeCl, CCl_4, 0 °C; (b) pyridine, 30% H_2O_2, 0 °C-room temperature (83%); (vi) acetone enol silyl ether, $ZnCl_2$, CH_2Cl_2, room temperature (64%); (vii) *m*-CPBA, $CHCl_3$, room temperature (82%); (viii) OsO_4 (cat.), $NaClO_3$, THF-H_2O (1:1), room temperature (93%); (ix) TsOH, benzene.

epoxide **9**, which forms a *trans*-diol **9a** as an intermediate of the ketalization reaction (step ix).

Treatment of *cis*-diol **8** with a catalytic amount of *p*-toluenesulfonic acid in hot benzene gave only the expected C-2′ *endo*-alcohol **10**, while similar treatment of epoxide **9** gave only the corresponding C-2′ *exo*-alcohol **11**. The ^1H NMR spectra of **10** and **11** showed the following notable differences. The benzylic C-1′ proton of exo isomer **11** appears as a doublet at δ 5.26 with a $J_{1'2'}$ = 1.9 Hz. The C-1 OH absorption, which appears as a sharp singlet at δ 13.12, is capable of hydrogen bonding only with the quinone carbonyl. The benzylic C-1′ proton of the endo isomer **10** occurs as a doublet of $J_{1'2'}$ = 4.95 Hz at δ 5.33, and the absorption for the C-1 OH, which is now capable of being doubly hydrogen bonded to the quinone carbonyl and to the endo C-2′ OH group, is shifted downfield to δ 13.37. The hydrogen bonding in compound **10** is additionally manifested by the higher R_f value (CH_2Cl_2) of **10** on silica gel G plates in comparison with compound **11**. The C-1′ proton absorptions reported for nidurufin and



10 **11**

6,8-dimethoxynidurufin are a doublet at δ 5.17 of $J_{1'2'}$ = 1.5 Hz and a doublet at δ 5.30 of $J_{1'2'}$ = 2.0 Hz, respectively. Reconsideration of these ^1H NMR data in the light of the data found for our unambiguously synthesized model compounds **10** and **11** suggests that nidurufin is actually *exo*-2′-hydroxyaverufin (**2a**)

(15) **7**: mp 122–123.5 °C; ^1H NMR ($CDCl_3$, 250 MHz) δ 2.20 (s, 3 H, $COCH_3$), 2.61 (m, 2 H, $CH_2CH_2COCH_3$), 2.67 (m, 2 H, $CH_2CH_2COCH_3$), 3.52 (s, 3 H, OCH_3), 5.39 (s, 2 H, OCH_2O), 6.66 (d, 1 H, J = 16.1 Hz, $ArCH{=}CHCH_2$), 6.85 (m, 1 H, $ArCH{=}CHCH_2$), 7.54 (s, 1 H, Ar H), 7.78 (m, 2 H, Ar H), 8.26 (m, 2 H Ar H), 13.58 (s, 1 H, ArOH). **6,8-Dideoxy-*endo*-2′-hydroxyaverufin (10)**: Purified by preparative TLC (R_f 0.5, CH_2Cl_2); mp 193–195 °C; ^1H NMR ($CDCl_3$, 250 MHz) δ 1.54 (s, 3 H, CH_3), (1.8–2.2 (m, 4 H, CH_2CH_2), 4.23 (m, 1 H, CH(OH), 5.33 (d, 1 H, J = 4.9 Hz, benzylic CH), 7.19 (s, 1 H, Ar H), 7.72 (m, 2 H, Ar H), 8.19 (m, 2 H, Ar H), 13.37 (s, 1 H, ArOH); mass spectrum, 352 (M^+, 31), 334 (20), 291 (13), 279 (19), 254 (41), 253 (29), 99 (100); MS (EI), m/z 352.0955 (352.0947 calcd for $C_{20}H_{16}O_6$). Anal. Calcd for $C_{20}H_{16}O_6$: C, 68.17; H, 4.58. Found: C, 67.83; H, 4.49. **6,8-Dideoxy-*exo*-2′-hydroxyaverufin (11)**: Purified by preparatie TLC (R_f 0.4, CH_2Cl_2); mp 189–190 °C; ^1H NMR ($CDCl_3$, 250 MHz) δ 1.64 (s, 3 H, CH_3), 1.8–2.2 (m, 4 H, CH_2CH_2), 4.06 (m, 1 H, CH(OH)), 5.26 (d, 1 H, J = 1.9 Hz, benzylic CH), 7.30 (s, 1 H, Ar H), 7.78 (m, 2 H, Ar H), 8.25 (m, 2 H, Ar H), 13.12 (s, 1 H, ArOH); mass spectrum, 352 (M^+, 5), 334 (27), 291 (16), 279 (21), 254 (90), 253 (30), 99 (100); MS (EI), m/z 352.0937 (352.0947 calcd for $C_{20}H_{16}O_6$). Anal. Calcd for $C_{20}H_{16}O_6$: C, 68.17; H, 4.58. Found: C, 68.00; H, 4.70. **Nidurufin**[5] **(2a)**: ^1H NMR (100 MHz, acetone-d_6) δ 1.58 (s, 3 H, CH_3), 3.97 (m, 1 H, CH(OH)), 5.17 (d, 1 H, J = 1.5 Hz, benzylic CH). **6,8-Dimethoxynidurufin**[6] **(2c)**: ^1H NMR δ 1.64 (s, 3 H, CH_3), 1.6–2.2 (m, 4 H, CH_2CH_2), 4.00 (s, 3 H, OCH_3), 4.04 (s, 3 H, OCH_3), 4.16 (7, 1 H, CH(OH)), 5.30 (d, 1 H, J = 2.0 Hz, benzylic CH), 6.86 (d, 1 H, J = 2.5 Hz, Ar H), 7.28 (s, 1 H, Ar H), 7.46 (d, 1 H, J = 2.5 Hz, Ar H).

and *not* the hydrogen-bonded *endo*-2′-hydroxyaverufin (**2b**) as previously assumed.

If a nidurufin-type intermediate is indeed involved in the averufin to versicolorin A transformation, it seems likely that this intermediate is the *exo*-alcohol nidurufin (**2a**) and not the unknown C-2′ endo isomer epinidurufin (**2b**), since only **2a** has the favorable stereochemistry for an AR_13-promoted rearrangement.

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Registry No. 2a, 28458-23-3; **5**, 518-83-2; **6**, 92762-95-3; **6**-ol, 92762-96-4; **6** (chloro derivative), 92762-97-5; **7**, 92762-98-6; **8**, 92762-99-7; **9**, 92763-00-3; **9a**, 92763-01-4; **10**, 92763-02-5; **11**, 92842-94-9; **5** (methoxymethyl ether), 64517-18-6; **5** (allyl ether), 92763-03-6.

Spin Multiplet Enhancement in Two-Dimensional Correlated NMR Spectroscopy

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Two-dimensional (2D) homonuclear correlated spectroscopy[1-4] is probably the most widely used 2D NMR experiment to date. It has proven to be a convenient and powerful method for tracing the pattern of homonuclear couplings in molecules with a molecular weight of up to 10000 daltons. However, it appears in practice that the intensity of a cross multiplet, indicating spin coupling, is not directly related to the magnitude of the homonuclear coupling involved. Sometimes protons that have a large scalar interaction show vanishing intensity for the cross multiplet. We propose a method for enhancing selectively the intensity of certain cross peaks of interest and for improving the sensitivity of the experiment.

From the recently introduced operator formalism approach,[5-7] it is easily found that the amount of magnetization transfer, R_1, from nucleus A to nucleus X in the COSY experiment has the proportionality

$$R_1(t_1) = \sin(\pi J_{AX} t_1) \prod_k \cos(\pi J_{Ak} t_1) \qquad (1a)$$

where k denotes the nuclei other than X to which A is coupled, and t_1 is the duration of the evolution period. Just after the mixing pulse, magnetization transferred from A to X is in antiphase with respect to spin A[2,5] and in phase with respect to other spins, n, to which X is coupled. The detected X spin magnetization has the t_2 (detection time) dependence:

$$R_2(t_2) = \sin(\pi J_{AX} t_2) \prod_n \cos(\pi J_{Xn} t_2) \qquad (1b)$$

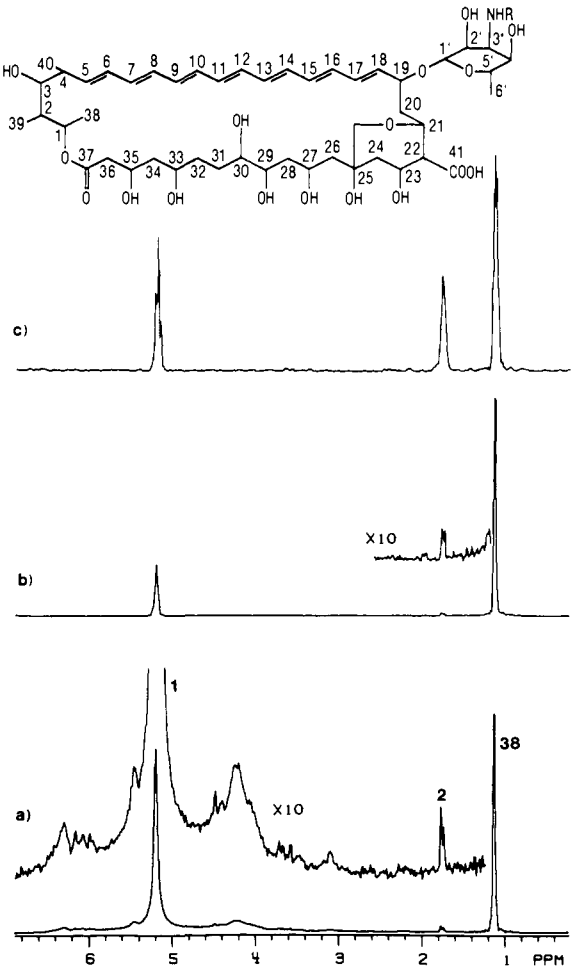

Figure 1. Cross sections parallel to the F_1 axis through the 2D COSY spectra of a sample of amphotericin B, recorded at 500 MHz. The cross sections are taken at the F_2 frequency of proton H1. (a) Cross section if no digital filtering is used, (b) cross section if a single sine bell is used in both dimensions, and (c) the cross section obtained if the optimized filter of eq 3 is used in order to emphasize the H1-H2 cross peak.

If the experiment is performed with phase modulation and n-type (coherence-transfer echo) selection,[8] the detected X spin magnetization, originating from A, is then given by

$$s_{AX}(t_1,t_2) = M_{0A}[R_1(t_1)R_2(t_2)\exp(-t_1/T_{2A})\exp(-t_2/T_{2X})] \times \exp(-i\Omega_A t_1)\exp(i\Omega_X t_2) \qquad (2)$$

where M_{0A} denotes the longitudinal A spin magnetization just before the first pulse of the COSY experiment, and T_{2A} and T_{2X} are the transverse relaxation times of A and X. From the terms within the square brackets in eq 2, it is seen that the magnetization transfer from A to X does not only depend on the magnitude of J_{AX} but also on the couplings between A and spins k and between X and spins n. Optimal signal to noise for the AX cross multiplet will be obtained for matched filtering, i.e., by multipyling the time domain signal with the function within the square brackets in eq 2. This function is specific for magnetization transferred from A to X and will be nonmatched filtering for other magnetization components. Consequently, diagonal components will be strongly attenuated by such a function, as are other cross peaks that show a different multiplet structure. It is clear from eq 2 that cross peaks between A and X will be hard to observe, if both A and X are coupled to a large number of other spins. However, by the use of a filtering function matched for that particular transfer, all other magnetization components are attenuated relative to the magnetization that contributes to the AX cross peak.

(1) Jeener, J. Ampere International Summer School, Basko Polje, Yugoslavia, 1971.
(2) Aue, W. P.; Bartholdi, E.; Ernst, R. R. *J. Chem. Phys.* **1976**, *64*, 2229.
(3) Bax, A.; Freeman, R.; Morris, G. A. *J. Magn. Reson.* **1982**, *42*, 164.
(4) Bax, A.; Freeman, R. *J. Magn. Reson.* **1981**, *44*, 542.
(5) Sorensen, O. W.; Eich, G. W.; Levitt, M. H.; Bodenhausen, G.; Ernst, R. R. *Progr. Nucl. Magn. Reson. Spectrosc.* **1983**, *16*, 163.
(6) Van de Ven, F. J. M.; Hilbers, C. W. *J. Magn. Reson.* **1983**, *54*, 512.
(7) Packer, K. J.; Wright, K. M. *Mol. Phys.* **1983**, *50*, 797.
(8) Nagayama, K.; Kumar, A.; Wüthrich, K.; Ernst, R. R. *J. Magn. Reson.* **1980**, *40*, 321.