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Assignment of the High-Field Resonances of a Gorgosterol Derivative through the Use of Autocorrelated Two-Dimensional ^{1}H NMR Spectroscopy

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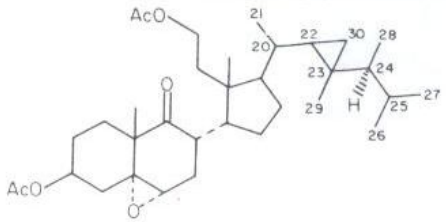
Since the initial report describing the novel cyclopropane-containing side chain of gorgosterol,[1] several additional steroids containing this interesting side chain have been reported.[2,3] The ^{1}H NMR spectra of all of these compounds exhibited signals for four high-field protons, although only three such protons would be expected in the trisubstituted cyclopropane moiety. In the specific case of gorgosterol, the protons at δ –0.13 and 0.44 were assigned as the cyclopropane methylene (H30) protons. One of the proton signals contained in the unresolved two-proton multiplet observed at δ 0.06–0.37 was further assigned as the remaining cyclopropane resonance (H22) on the basis of homonuclear decoupling experiments. The identity of the remaining proton signal contained in this multiplet has, however, never been accounted for, although it was suggested that that signal must be due to an unusually shielded proton not directly attached to the cyclopropane ring. We have performed an NMR study of a secogorgosterol derivative, 5α,6α-epoxy-3,11-dihydroxy-9,11-seco-5α-gorgostan-9-one 3,11-diacetate (1), which also exhibits four-proton resonances that correspond closely to those decribed in the initial report on the structure of gorgosterol.[1] Through the use of conventional and autocorrelated two-dimensional ^{1}H NMR spectroscopy (COSY),[4,5] the identities of all four of the upfield resonances have now been assigned and form the basis for this report.



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The diacetate 1 was obtained as a chromatographically pure oil by acetylation of the naturally occurring parent diol, which was isolated from *Pseudopterogorgia americana* (Gmelin, 1791) collected in the Florida keys. Both the parent diol and the corresponding diacetate were identical with compounds previously reported by Spraggins.[6]

The conventional ^{1}H NMR spectrum taken in deuteriochloroform at 360 MHz contained four multiplets in the upfield region of the spectrum with chemical shifts of δ 0.39, 0.17, 0.13 and –0.22, each accounting for one proton and comparing favorably with the chemical shifts reported for gorgosterol.[1] The resonances at δ 0.39 and –0.22 each appeared as a doublet of doublets (J = 4.4, 9.1, and J = 4.4, 5.9 Hz, respectively) and were assigned as the cyclopropane methylene (H30) resonances as in the previous study. The remaining protons exhibited shifts of δ 0.17 and 0.13 and appeared as a partially overlapped doubled quartet (J = 6.9, 8.7 Hz) and a doubled triplet (J = 5.9, 8.8 Hz), respectively. Structures of the two multiplets were confirmed by homonuclear two-dimensional J-resolved (2DJ) spectroscopy. The autocorrelated two-dimensional proton spectrum (COSY) of the diacetate (Figure 1) contained off-diagonal peaks 30/22[7] and 22/30 that confirmed that the double triplet (δ 0.13, H22) was coupled to the two cyclopropane methylene protons (H30), confirming the previous studies.[1,6] This resonance was also coupled

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(7) Identification of the off-diagonal peaks in the COSY spectrum is made by listing the identities of the individual protons, that resonating further downfield listed first. Those sets of off-diagonal peaks denoted by # are permutable assignments.

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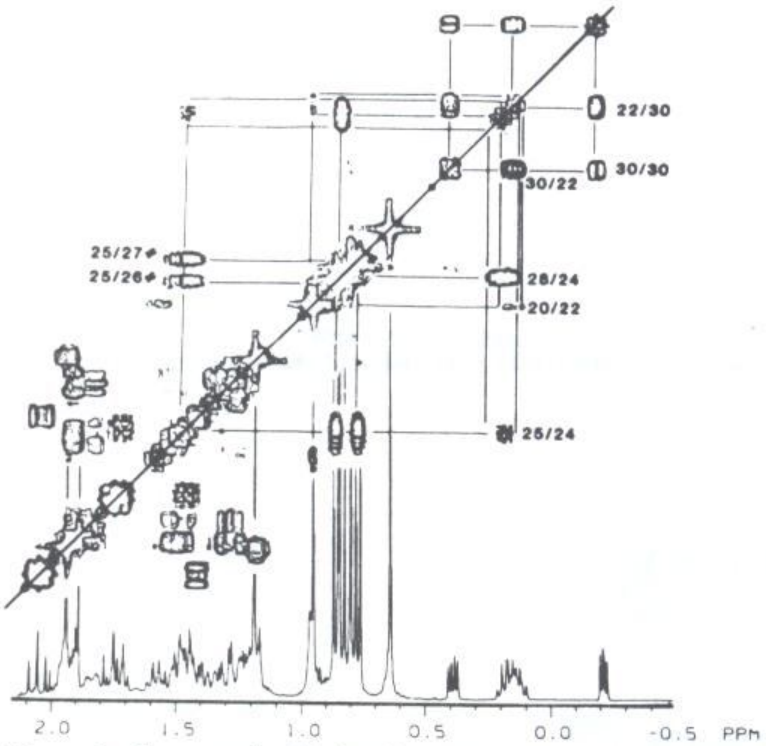

Figure 1. Contour plot (six level) of the autocorrelated (COSY) two-dimensional proton NMR spectrum (symmetrized[15]) of 1 in deuteriochloroform at 360 MHz. The final $\mathbf{S}(F_2,F_1)$ matrix plotted consisted of 512 × 512 data points. Off-diagonal responses establishing proton spin-coupling connectivities are labeled with the protons involved, the downfield resonance listed first.

(20/22) to a multiplet resonating at δ 0.96 (H20), which was partially obscured by a methyl singlet resonance (Me-21).

The resonance of the non-cyclopropane proton at δ 0.17 can be seen to be coupled (Figure 1) to a methyl doublet (28/24) located at δ 0.85 (J = 6.9 Hz) and further to a complex multiplet (25/24) resonating at approximately δ 1.47. The latter multiplet can further be seen to be coupled to two methyl doublets, 25/26#[7] and 25/27# resonating at δ 0.83 and 0.76 (J = 7.0 Hz in each case). The proton spin-coupling network established through the COSY experiment thus identifies the anomalous high-field proton as H24, the eight-line pattern arising from nonequivalent couplings to the H25 methine and H28 methyl protons. The further couplings of the former resonance, also established by the COSY experiment, identify the 26- and 27-methyl doublets, thus completing the assignment of all of the proton resonances of this complex side chain.

Corroboration of the assignment of the resonance observed at δ 0.17 to H24 follows from consideration of the first-order character of this resonance and that at δ 0.13. The sole alternative proton that might account for the signal at δ 0.17 would be the 20-methine proton. In this case, we would expect the muliplet to be non-first-order due to strong coupling between the H20 and H22 resonances even at 360 MHz. The highly first-order character of these multiplets, however, and the coupling network established through the COSY experiment clearly precludes the assignment of the resonance at δ 0.17 to H20, thus confirming the assignment of this resonance as H24.

The dramatic shielding of H24 is undoubtedly caused by the steric demands of this very highly substituted side chain. The preferred conformations thus imposed are also reflected by the unusually large vicinal couplings displayed by the H22 (8.8 Hz) and H24 (8.7 Hz) protons on the side-chain backbone.

In summary, the utilization of two-dimensional autocorrelated ^{1}H NMR (COSY) spectra[4,5,8,9] or alternatively SECSY spectra,[10-13] promises to provide an extremely powerful tool for natural-products structure elucidation. Work is presently in progress in these laboratories to apply these techniques to other novel marine natural products, the results of which will be forthcoming.[14]

Experimental Section

The COSY spectrum utilized in this study was obtained by using an initial $\mathbf{S}(t_1,t_2)$ data matrix consisting of 1024 × 512 data points, the data acquired by using phase cycling of the second 90° pulse to provide the equivalent of quadrature detection in both time domains. Data presented in Figure 1 are shown as a six-level contour plot symmetrized after the second Fourier transformation.[15] All spectra were taken in deuteriochloroform on a sample prepared by dissolving 40 mg of 1 in approximately 0.5 mL of solvent. Spectra were taken at 361.053 MHz on a Nicolet WB-360 spectrometer. The proton reference spectrum plotted below the contour plot of the COSY data was obtained by using 32K data points.

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Registry No. 1, 86853-44-3.

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