Protein Plasticity: A Single Amino Acid Substitution in the *Saccharomyces cerevisiae* Oxidosqualene-Lanosterol Cyclase Generates Protosta-13(17), 24-dien-3β-ol, a Rearrangement Product

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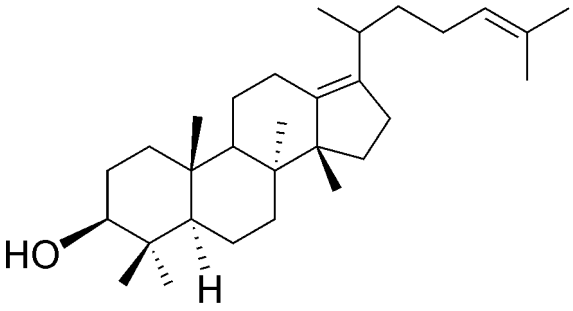
Experimental Procedure

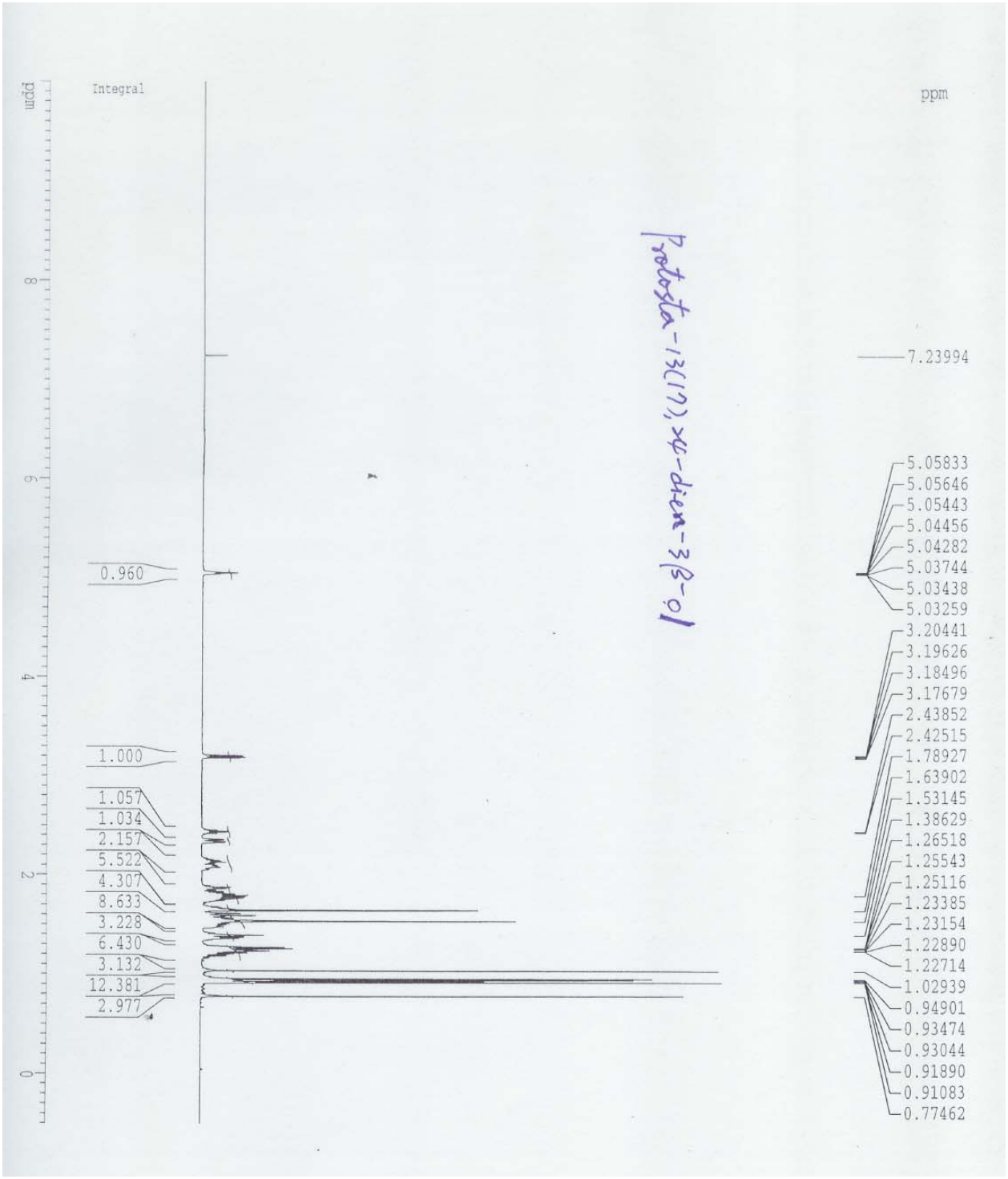
Generation and Analysis of Mutant Extracts. Mutagenesis of Phe699 in the *S. cerevisiae* ERG7 wild-type gene was performed using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA). The degenerate mutagenic primers for Phe699 substituted with Ser, Gln, Cys, Tyr, and Thr were the following, with substitutions underlined and silent mutation italicized: ERG7F699SQCYT1: 5'-d(GTGTAGAAGGTGTT(A/T/C)(A/C/G)(A/C)AACCA*CAGCTG*TGCAATTG)-3' and ERG7SQCYT2: 5'-d(CAATTGCA*CAGCTG*TGGTT(G/T)(T/C/G)(A/T/G)AACACCTTCTACAC)-3'. The PCR reaction contains 0.8 mM each of dNTP, 100 ng of pRS314ERG7WT plasmid as template, 1X *Pfu* polymerase buffer, 10 μL of each primer, 2.5 U of *Pfu* DNA polymerase and ddH_2O to the final volume of 50 μL. The reaction mixture was denatured at 95 °C for 2 min, and then run for 18 cycles of denaturizing at 95 °C for 30 sec each, annealing at 53 °C for 1 min, polymerization at 68 °C for 1 min, and a final extension at 68 °C for 16 min. The mutations were confirmed by DNA sequencing and subsequently electroporated into the yeast strain TKW14 and selected for growth on SD+Ade+Lys+His+ Met+Ura+hemin+G418+Erg plates. The plasmids were then selected on SD+Ade+Lys+His+Met+Ura+hemin+G418+5-FOA plates for complementation of cyclase activity as described previously. Transformants were grown in SD+Ade+Lys+His+Met+Ura+hemin +G418+Erg medium for non-saponifiable lipid (NSL) extraction and column chromatography. The NSL extract was fractionated by silica gel column chromatography and assayed by gas chromatography-mass spectrometry (GC-MS) to examine triterpenoid products with a molecular mass of m/z = 426 as described previously.

Chemical Shifts of protosta-13(17),24-dien-3β-ol: The ^{1}H NMR spectra showed one olefinic proton (δ 5.044), two vinylic methyl signals (δ 1.639, 1.531), five methyl singlets (δ1.029, 0.949, 0.935, 0.911, 0.775), and one methyl doublet (δ 0.925, d, J = 6.9 Hz, 3H). The 150 MHz ^{13}C NMR spectrum revealed the presence of one tertiary-quaternary (δ = 124.96, 130.90 ppm) and one quaternary-quaternary substituted double bond (δ = 135.23, 138.98 ppm). The HMQC spectrum showed that the methyl doublet protons at δ = 0.925 were attached to the carbon at 20.05 ppm, the methine proton at δ 2.432 was attached to the carbon at 31.56 ppm (C-20), while the methylene protons at δ 2.336 and 1.779 were attached to the carbon at 23.24 ppm (C-12). In the HMBC spectrum, the δ 2.432 methine proton is coupled by ^2J to carbons at 135.23 ppm (C-17), 35.64 ppm (C-22), and 20.05 ppm (C-21), as well as by ^3J to carbons at 138.98 ppm (C-13), 28.65 ppm (C-16), and 26.32 ppm (C-23). The HMBC also established that the tertiary vinylic proton (δ 5.044) was coupled by ^2J to carbons at 130.94 ppm (C-25) and 26.32 ppm (C-23), as well as by ^3J connectivity to carbons at 25.71 ppm (C-26), 17.58 ppm (C-27), and 35.64 ppm (C-22).

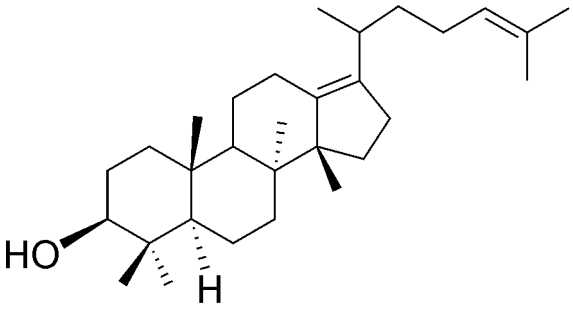
Chemical shifts were referenced to $Si(CH_3)_4$ and are generally accurate to +0.01 ppm. ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.044 (t, J = 7.0 Hz, 1H, H-24), 3.191 (dd, J = 11.6, 4.9 Hz, 1H, H-3α), 2.432 (m, 1H, H-20R), 2.336 (dddd, J = 15.1, 4.9, 1.6, 1.6 Hz, 1H, H-12β), 2.069-2.140 (m, 2H, H-16), 1.737-1.871 (m, 5H, 1H for H-7β, 1H for H-12α, 1H for H-15β, 2H for H-23), 1.652-1.667 (m, 1H, H-2α), 1.639 (s, 3H, Me-26), 1.609 (d, J = 7.3 Hz, 1H, H-9β), 1.585-1.590 (m, 1H, H-2β), 1.471-1.568 (m, 3H, 1H for H-1β, 1H for H-6α, 1H for H-11α), 1.531 (s, 3H, Me-27), 1.348-1.408 (m, 2H, 1H for H-1α, 1H for H-5α), 1.166-1.280 (m, 6H, 1H for H-6β, 1H for H-7α, 1H for H-11β, 1H for H-15α, 2H for H-22), 1.029 (s, 3H, Me-18), 0.949 (s, 3H, Me-29), 0.935 (s, 3H, Me-19), 0.925 (d, J = 6.9 Hz, 3H, Me-21), 0.911 (s, 3H, Me-30), 0.775 (s, 3H, Me-28). ^{13}C NMR Spectra δ 33.82 (C-1), 28.88 (C-2), 79.22 (C-3), 39.28 (C-4), 47.58 (C-5), 18.35 (C-6), 33.31 (C-7), 40.08 (C-8), 47.18 (C-9), 36.80 (C-10), 23.03 (C-11), 23.24 (C-12), 138.98 (C-13), 57.40 (C-14), 30.93 (C-15), 28.64 (C-16), 135.23 (C-17), 22.58 (C-18), 23.94 (C-19), 31.56 (C-20), 20.05 (C-21), 35.64 (C-22), 26.32 (C-23), 124.96 (C-24), 130.90 (C-25), 25.71 (C-26), 17.58 (C-27), 15.97 (C-28), 28.85 (C-29), 22.89 (C-30).

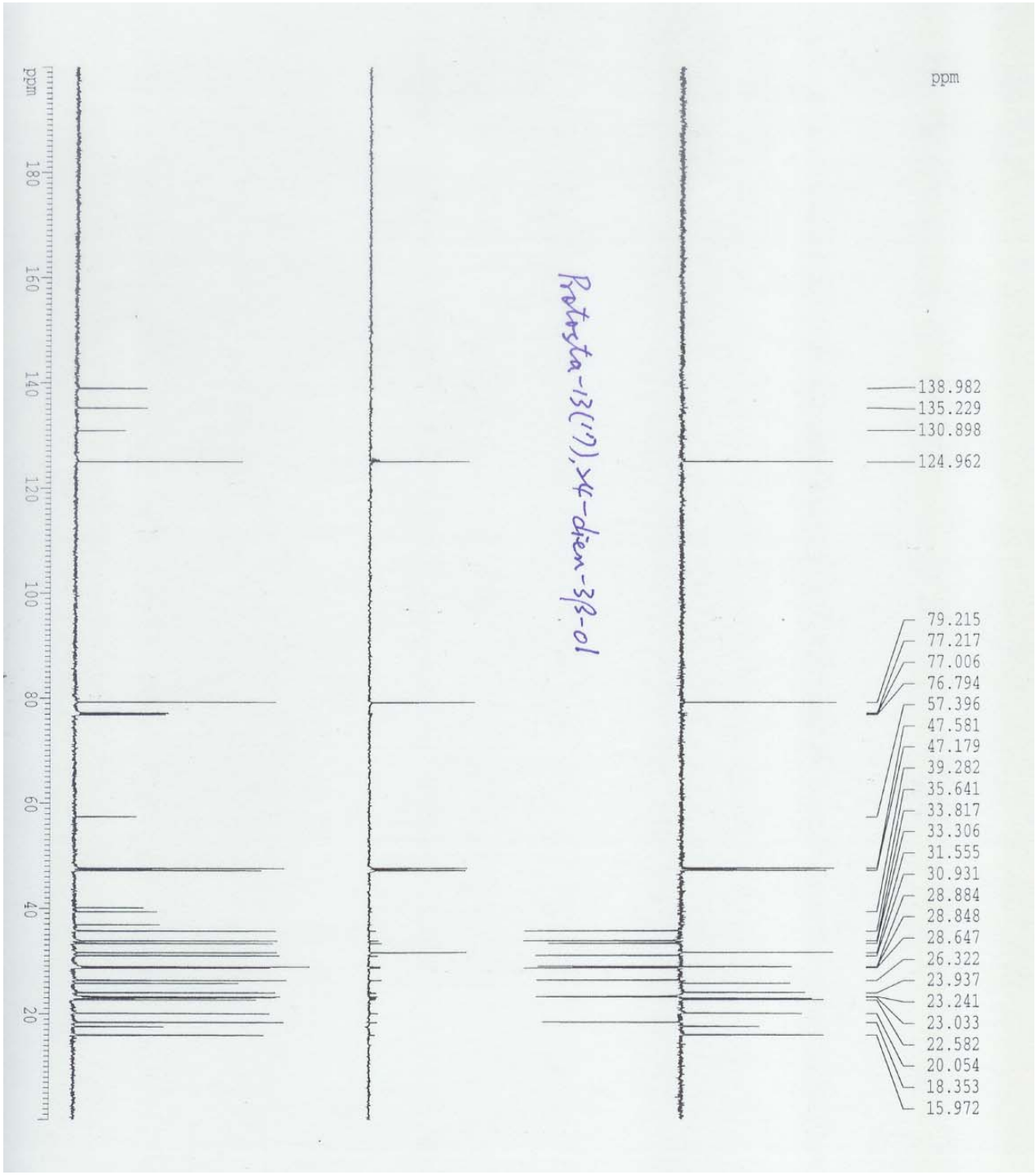
[1]H-NMR spectrum of Protosta-13(17),24-dien-3β-ol, **3**



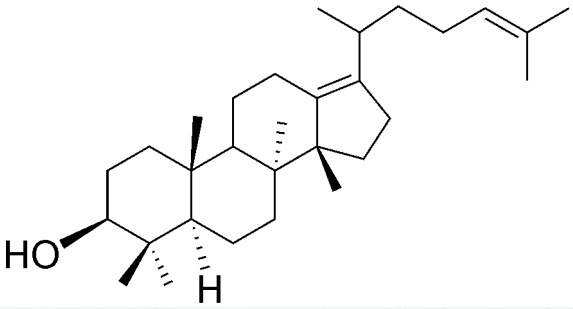


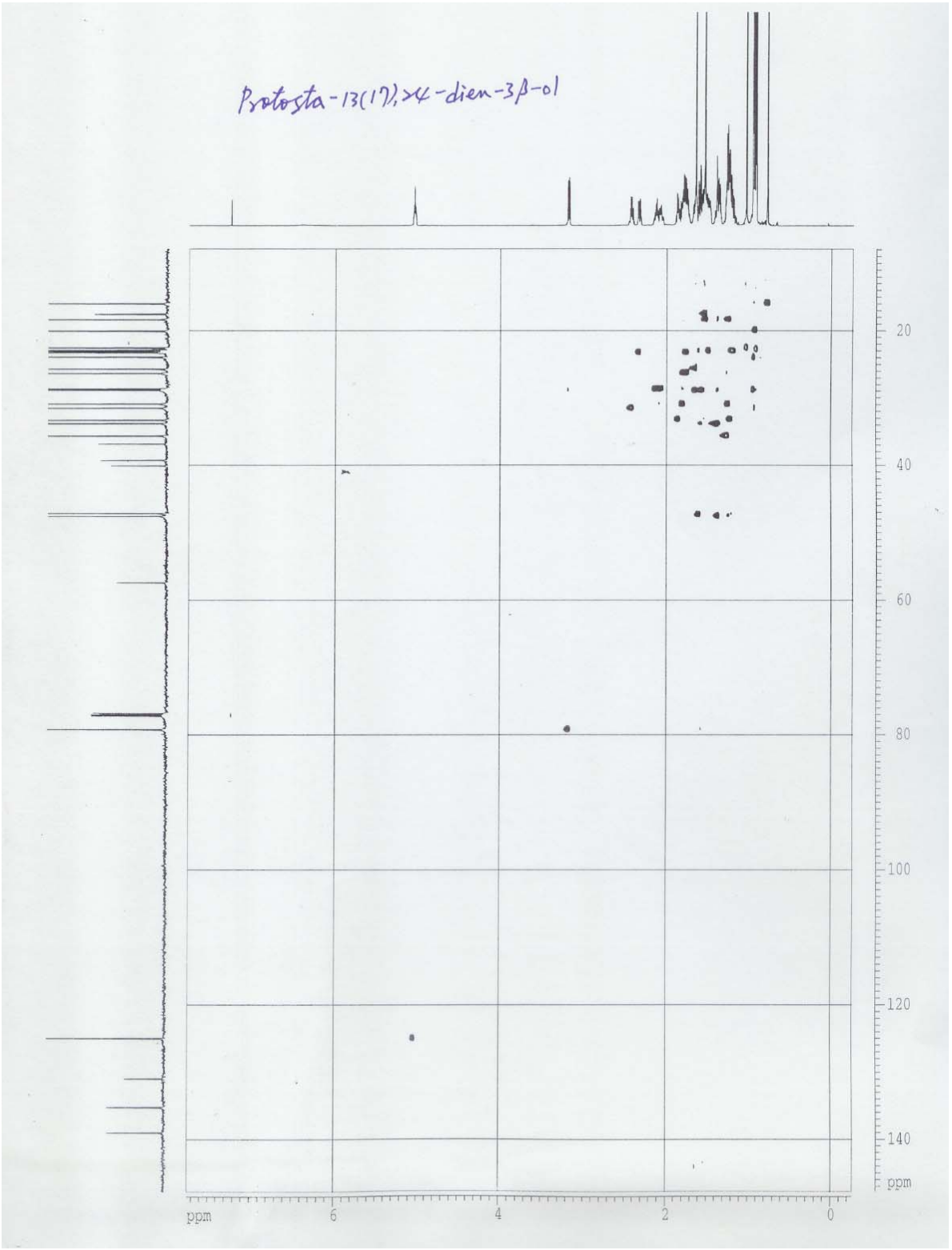
^{13}C-NMR and DEPT spectra of Protosta-13(17),24-dien-3β-ol, **3**



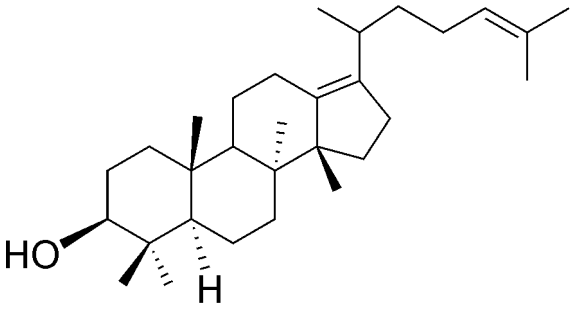


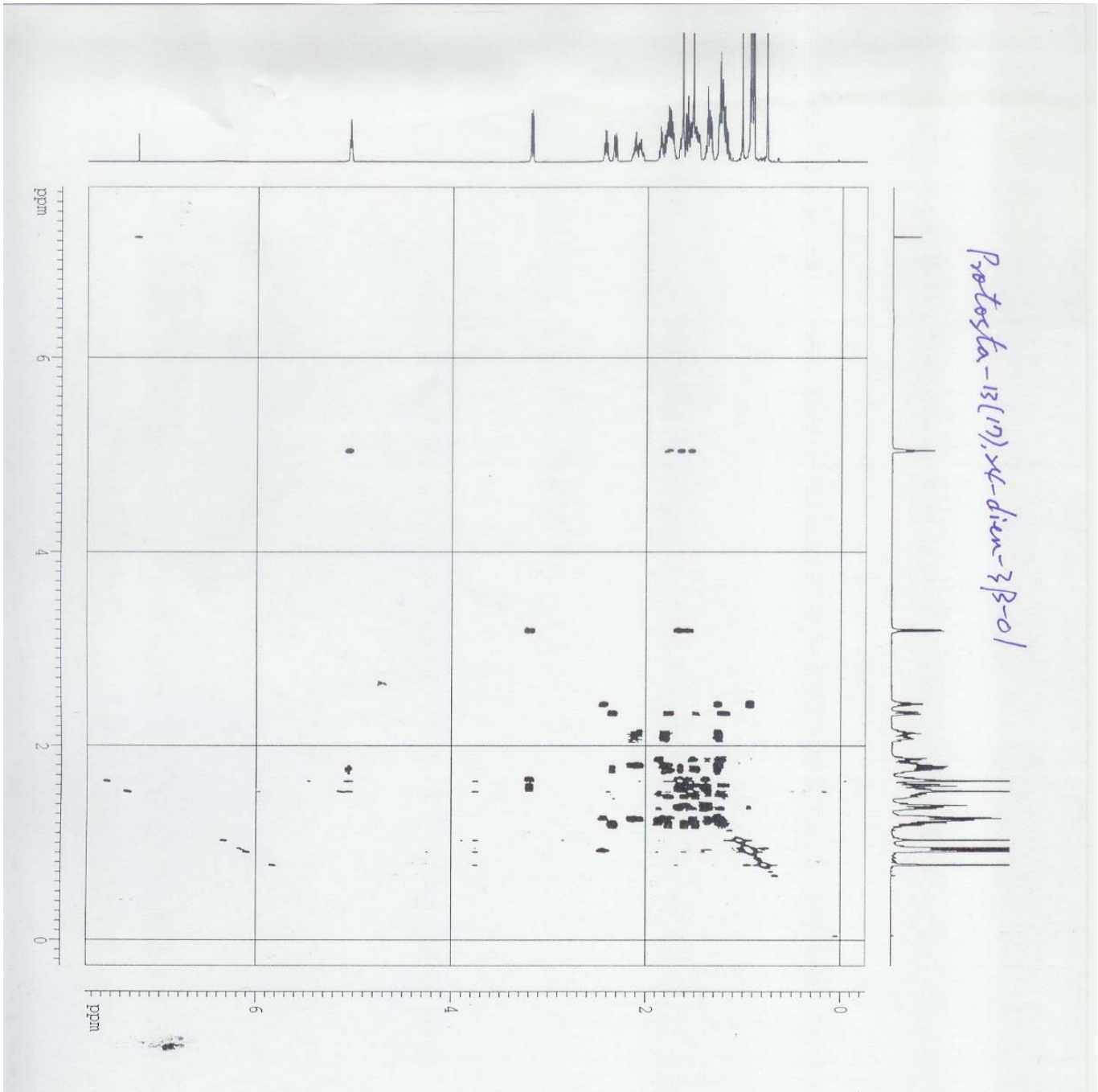
HMQC of Protosta-13(17),24-dien-3β-ol, **3**





^{1}H -^{1}H COSY of Protosta-13(17),24-dien-3β-ol, **3**





HMBC of Protosta-13(17)24-dien-3β-ol, **3**