Electronic Supplementary Information

Supplementary Material (ESI) for CrystEngComm

Fifty-year old samples of progesterone demonstrate the complex role of synthetic impurities in stabilizing a metastable polymorph

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Contents

1. Reference IR spectra of Progesterone forms 1 and 2.

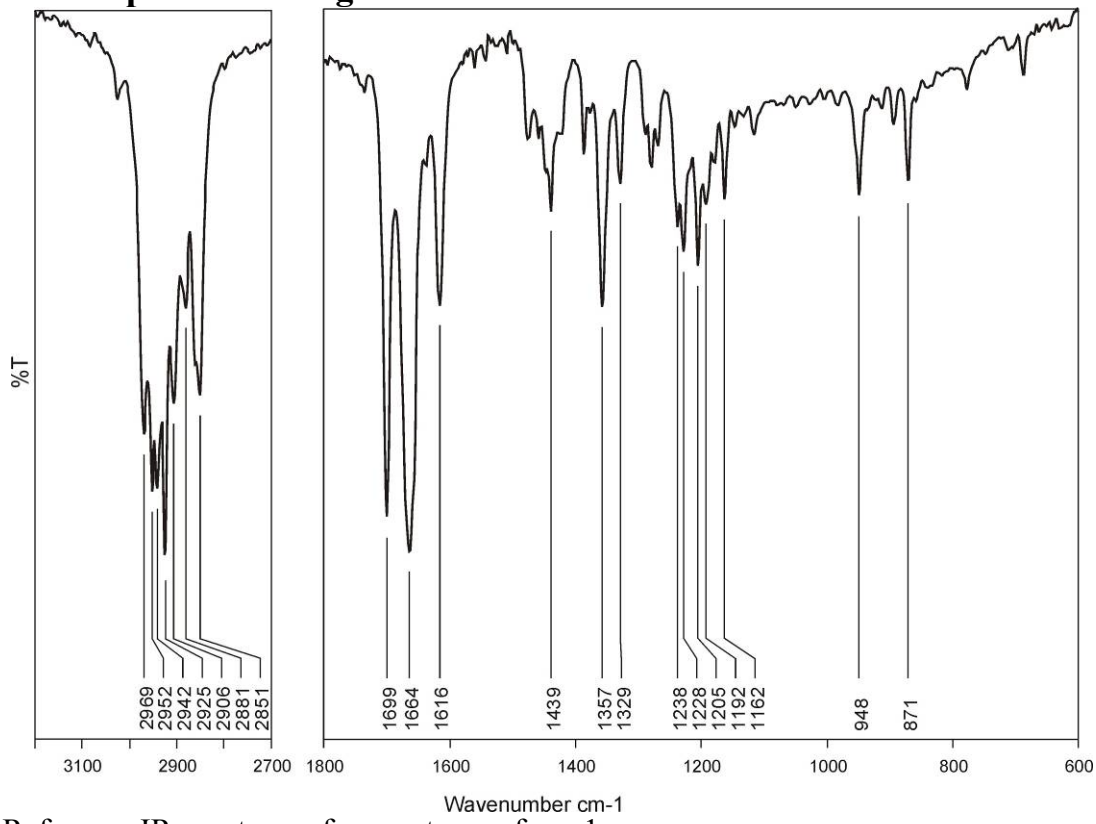

Figure S1. Reference IR spectrum of progesterone form 1.

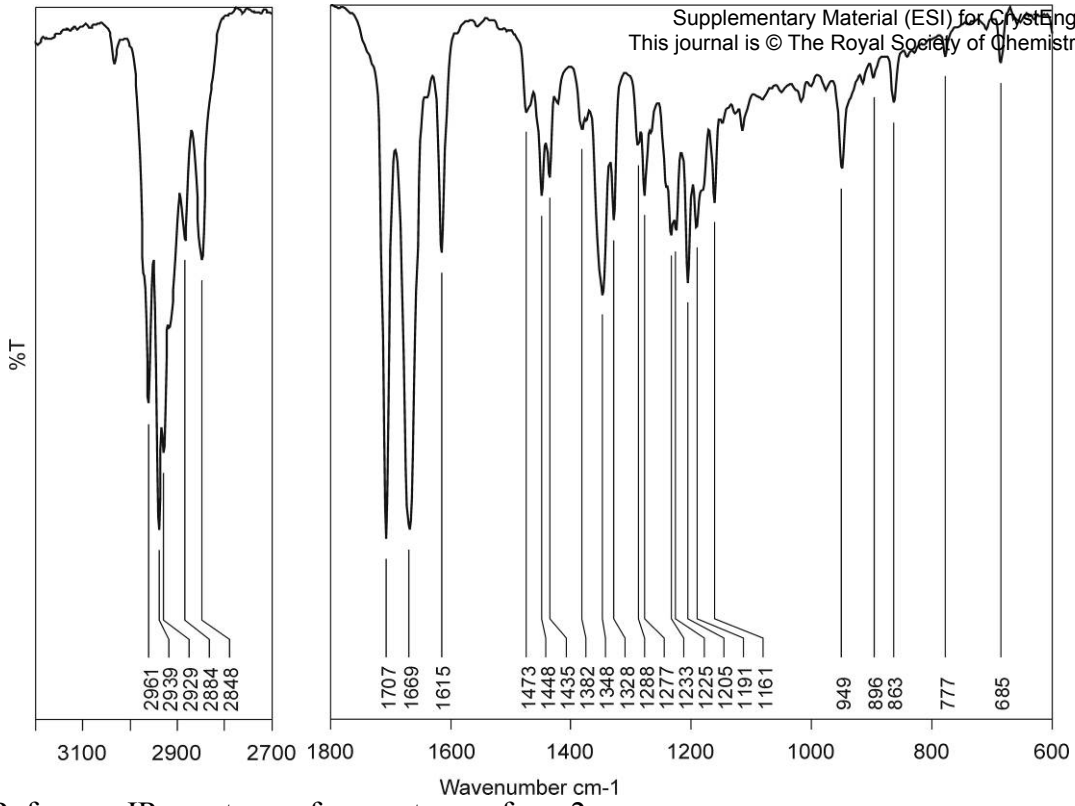

Figure S2. Reference IR spectrum of progesterone form 2.

2. Details of polymorph nomenclature associated with the progesterone literature and associated documented crystal structures.

Polymorph nomenclature / notation				CSD refcodes
Form 1 Refs: [1,2]	Form I, high temperature phase M.pt. 129 °C Refs: [3-7]	α form Refs: [8,9]	Form A Ref: [10]	PROGST10[3] PROGST03[4] PROGST02[7] PROGST12[11]
Form 2 Refs: [1,2]	Form II, low temperature phase M.pt. 122 °C Refs: [5,6,12,13]	β form Refs: [8,9]	Form B Ref: [10]	PROGST01[14] EWACUA[13] (*ent* progesterone) PROGST13[11]

References

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13. R. J. Auchus, A. S. Kumar, C. A. Boswell, M. K. Gupta, K. Bruce, N. P. Rath and D. F. Covey, *Arch. Biochem. Biophys.*, 2003, 409, 134-144.
14. E. Foresti Serantoni, A. Krajewski, R. Mongiorgi, L. Riva de Sanseverino and R. Cameroni, *Cryst. Struct. Commun.*, 1975, 4, 189-192.

3. Mass spectrum of form 1 conditions used for obtaining LC-MS data and information about molecular ions found.

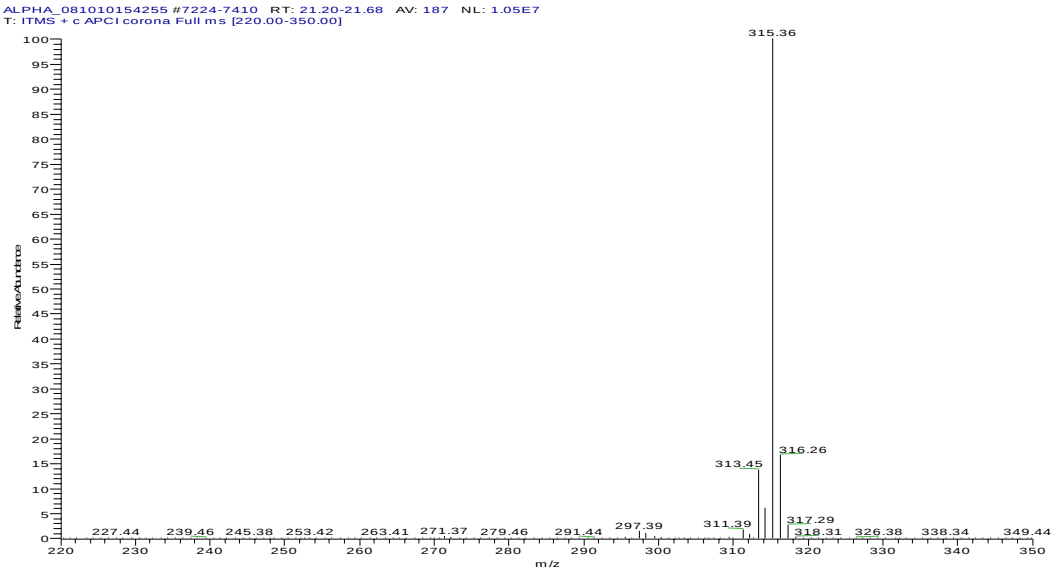

Figure S3. Mass Spectrum (APCI) of progesterone form 1 (the mass spectrum of form 2 bears a close resemblance to form 1).

LC-MS data was collected on a **Thermo Finnigan LTQ Ion-Trap** operating in Atmospheric Pressure Chemical Ionization (APCI) positive mode equipped with an **Agilent 1100 series LC**. The LC conditions were as follows: **Column**: Agilent Eclipse XDB-C18 150 mm x 4.6 mm, **Flow**: 0.5 mL/min, **Mobile Phase**: (Isocratic) 50:50 MeCN:H_2O (0.25Mm NH_4OAc), **Injection Volume**: 10µL. The Mass Spectrometer conditions were as follows: **Mode**: APCI+, **Vaporizer Temperature (°C)**: 475, **Capillary Temperature (°C)**: 275, **Tube Lens (V)**: 80, **Sheath Gas Flow Rate (arb)**: 40, **Aux Gas Flow Rate (arb)**: 5, **Sweep Gas Flow Rate (arb)**: 5, **Discharge Current (µA)**: 5.

The molecular ions associated with some of these components are $[M+H]^+$ = 288.9, 331.2, 329.2, 317.2, 313.2, and 315.2. The last corresponds to progesterone and 317.2 appears to correspond to pregnenolone. However, if a sample of progesterone is spiked with pregnenolone it co-elutes with the former, suggesting that $[M+H]^+$ = 317.2 is an isomer of pregnenolone. The possibility of an isomer of pregnenolone present as an impurity in pregnenolone when used in the early syntheses has been considered. Plausible identification of some of the remaining impurities are as follows: $[M+H]^+$ = 331.2 - hydroxyprogesterone (11-OH or 17-OH?); $[M+H]^+$ = 329.2 – bisnoraldehyde (from ergosterol?); $[M+H]^+$ = 313.2 - 1- dehydroprogesterone. The latter is considered to be a potential impurity present at a level at greater than 0.1% whilst hydroxyprogesterone, if present, would probably exist at levels below 0.1%.[1] It should be stressed that there is absolutely no inference that any modern synthesis corresponds to that used to produce the 50-year old Innsbruck sample, indeed we feel that to be a very unlikely scenario.

Reference.

1. Progesterone USP Regulatory filing. PfizerCentreSource.com, 2004.

4. Conditions used for obtaining solution state NMR spectra.

NMR spectra were obtained using a Bruker DRX400 system with a 5 mm DUL $^{1}H/^{13}C$ probe under ambient conditions. The probe was tuned and matched for all samples prior to data acquisition and referenced to the residual solvent signal of $CDCl_3$.

5. Solution state NMR spectrum of progesterone (for assignment and reference purposes), plus spectrum of form 2 with impurities highlighted.

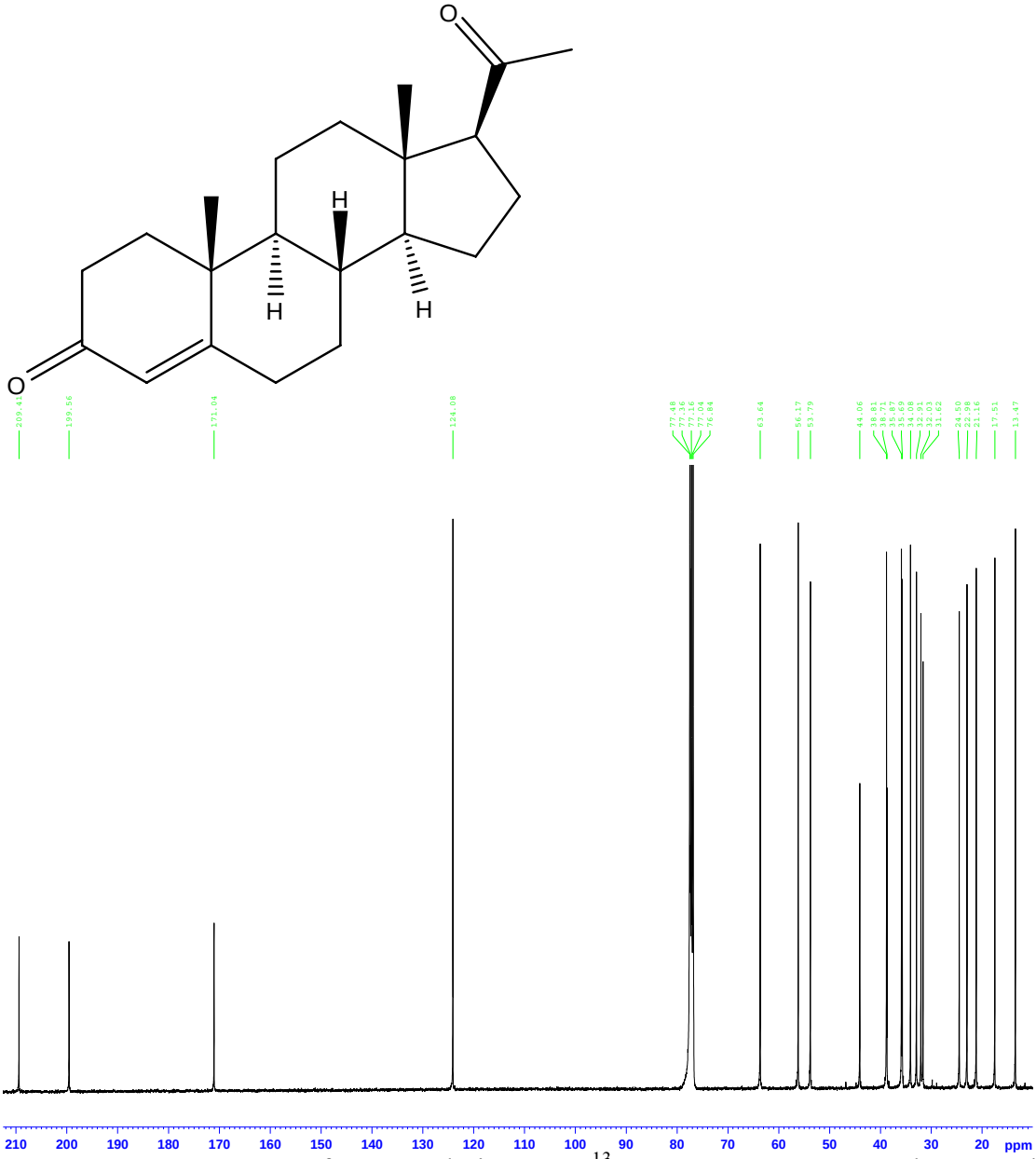

Figure S4. Progesterone form 2 solution-state ^{13}C NMR. Progesterone shows peaks at (Assignments in brackets) δ 209.4 (C20), 199.6 (C3), 171.0 (C5), 124.1 (C4), 63.6 (C17), 56.2 (C14), 53.8 (C9), 44.1 (C13), 38.8, 38.7 (C12, C10), 35.9, 35.7, 34.1 (C1, C8, C7), 32.9, 32.0 (C2, C6), 31.6 (C21), 24.5, 23.0, 21.2, (C15,11,16), 17.5 (C19), 13.5 (C18).

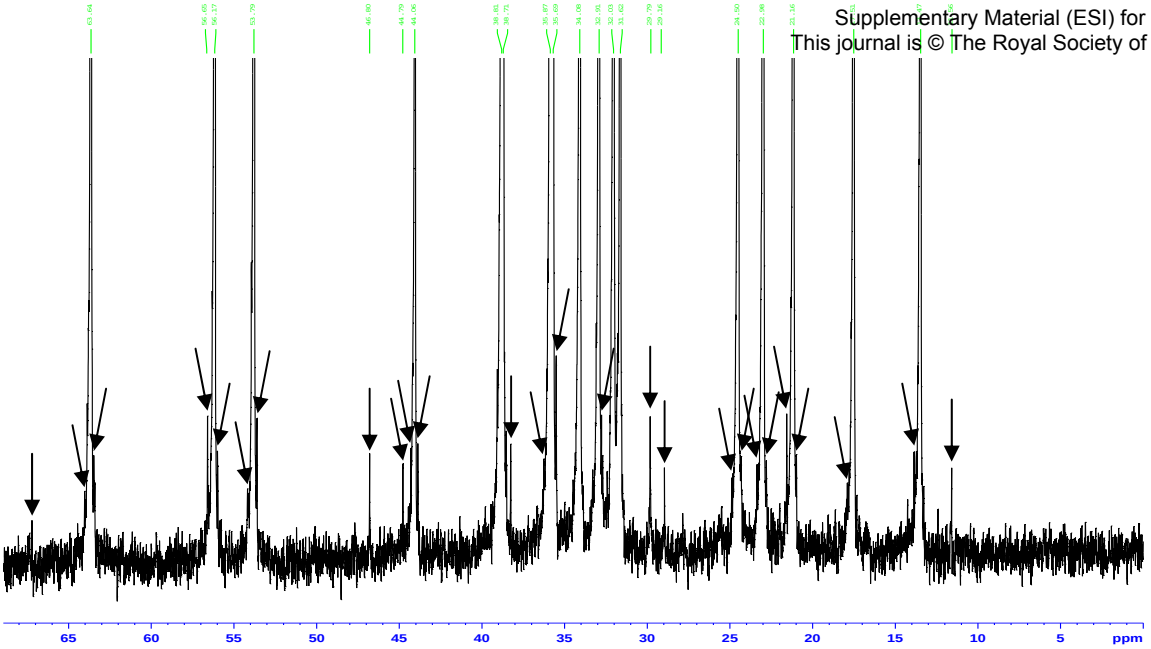

Figure S5. Expanded spectrum of form 2 with carbon signals associated with impurities arrowed. The spectrum of form 1 is identical, though on a similar scale of expansion there is no obvious evidence for impurities.

The proton spectrum of form 2 did not offer any evidence for impurities but the ^{13}C spectrum of the metastable form 2 (Figure S2) showed evidence for trace impurity(s) with extra signals, possibly associated with skeletal carbon atoms proximal to the junction of the B and C rings. Alternatively, the impurity peaks could be attributed to a fairly simple aliphatic compound, though there is no evidence to suggest that such a compound would be associated with the synthesis, or if it were, carried through it. The S/N associated with the spectrum was poor owing to the number of low level impurities present. There were no obvious impurities present in the spectrum of form 1.

6. Impurity profile for Aldrich reference material.

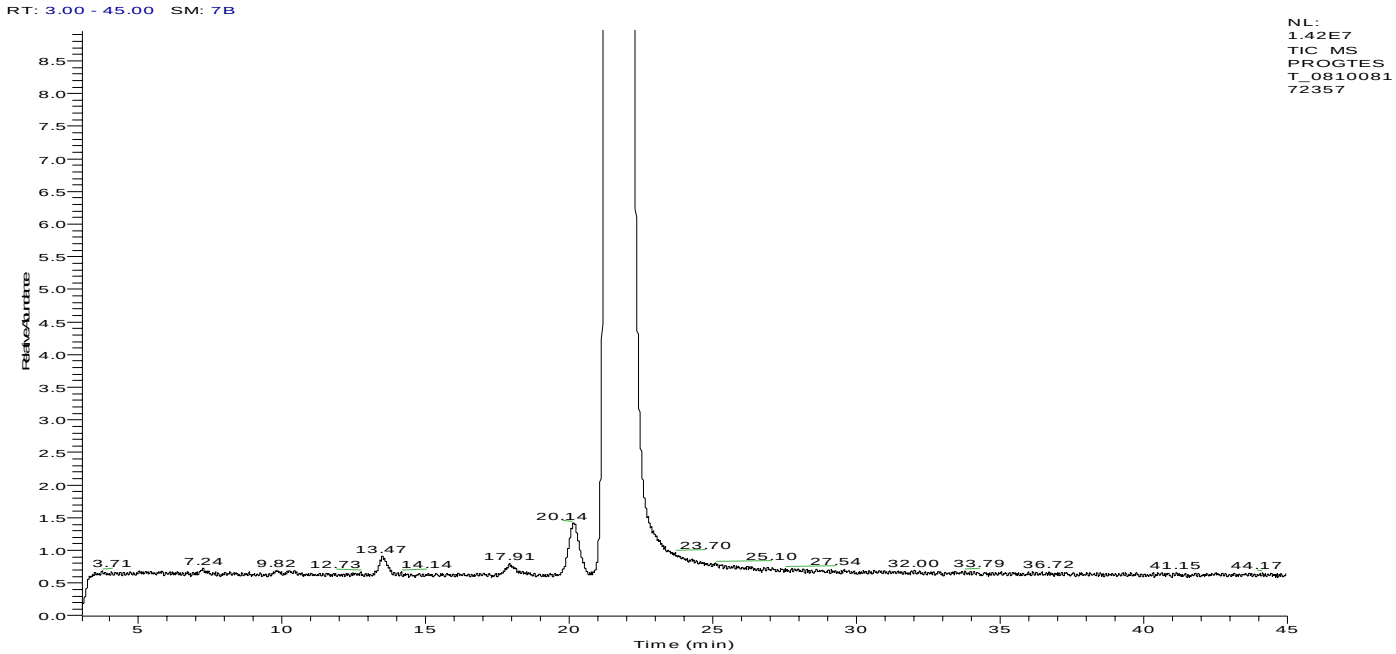

Figure S6. LC trace for Aldrich reference material (1.2% impurities).

7. Origin of reference samples used in characterization and spiking experiments in conjunction with NMR and LC-MS analysis.

Samples of 16-dehydroprogesterone, 4-Pregnen-20β-ol-03-one, 4,6-Pregnadiene-3,20-di-one, 4-Pregnen-3-one-20-β–carboxyaldehyde, and 4-Pregnen-20α-ol-3-one were obtained from Steraloids and used as reference standards.

8. LC-MS spiking experiments with reference standards.

From the Mass Spec data several potential steroids were considered. LC traces and Mass Spectra for each are shown below.

PROGESTERONE (PURE)

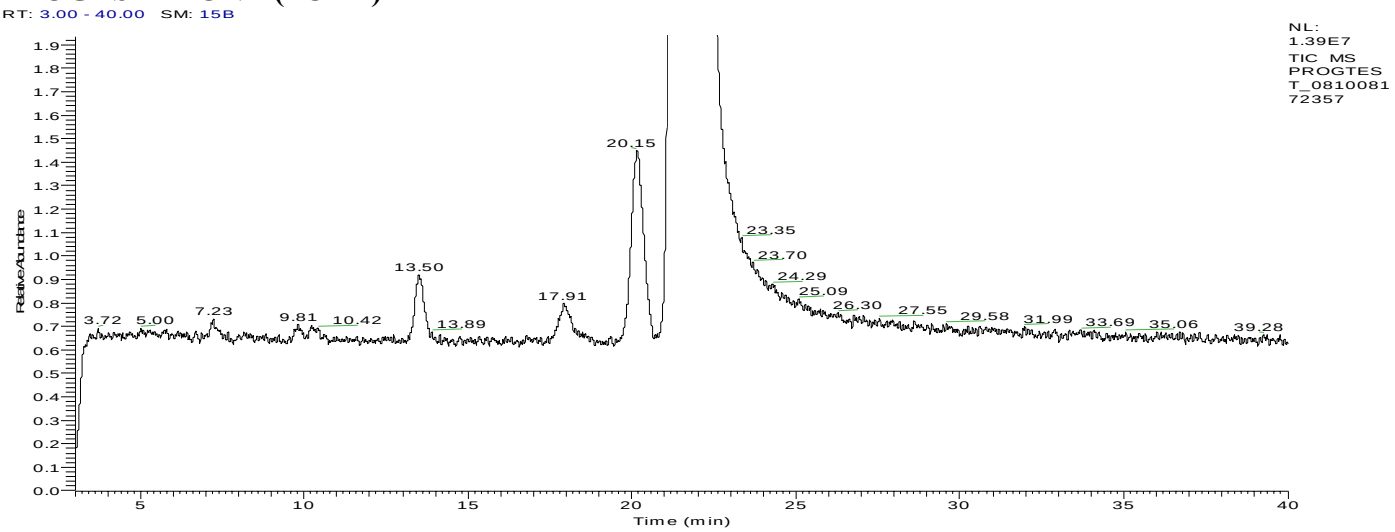

Figure S7. Expanded LC trace for progesterone (pure).

Peaks are seen at 7.23, 9.81, 10.42, 13.5, 17.91 and 20.15 min (peak at 21.4 min being progesterone). Peaks at 7.23, 9.81, 10.42 and 17.91 are also seen in the 'beta-progesterone' LC and have the same mass spectra.

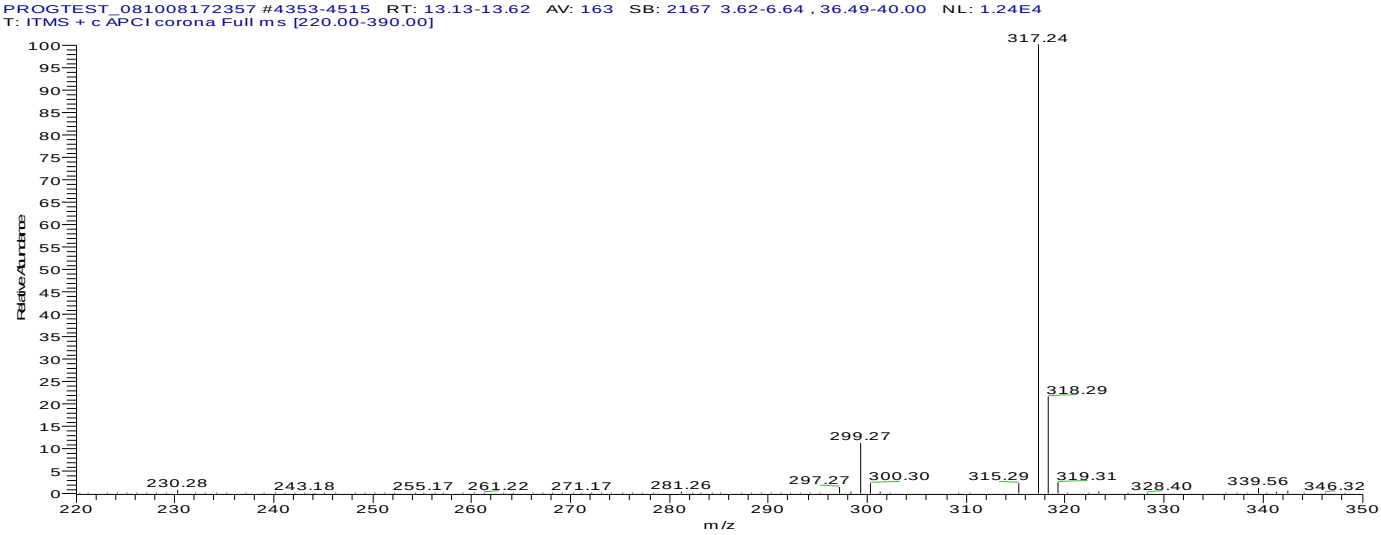

Figure S8. Mass Spectrum of peak at 13.5 min.

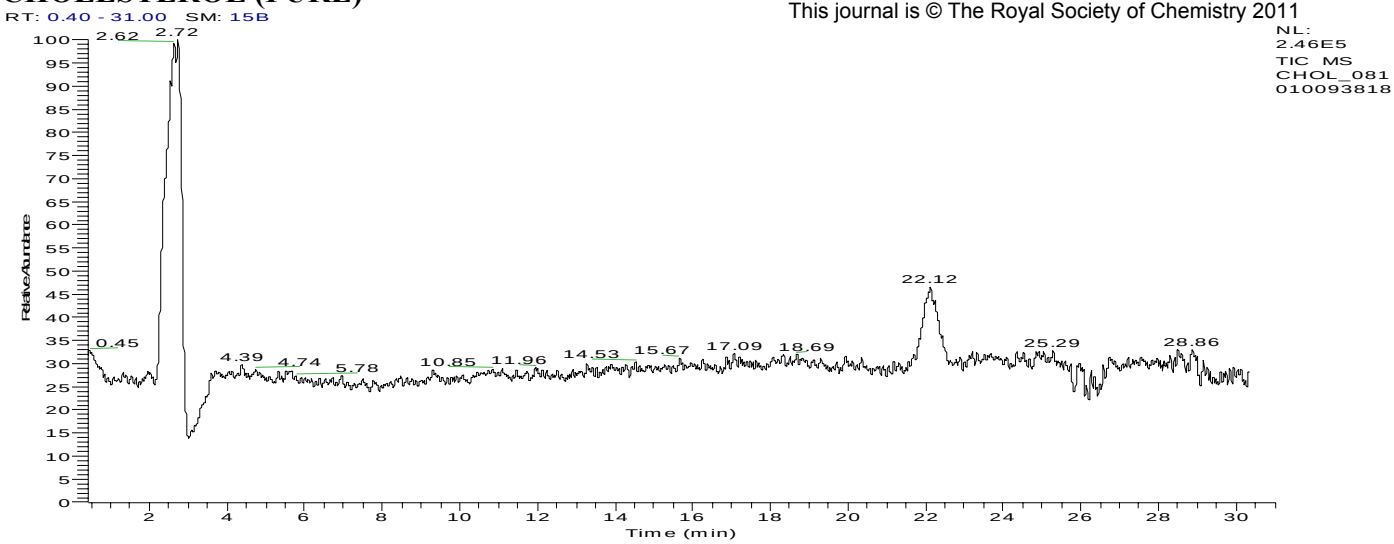

Figure S9. LC Trace for Cholesterol (pure).

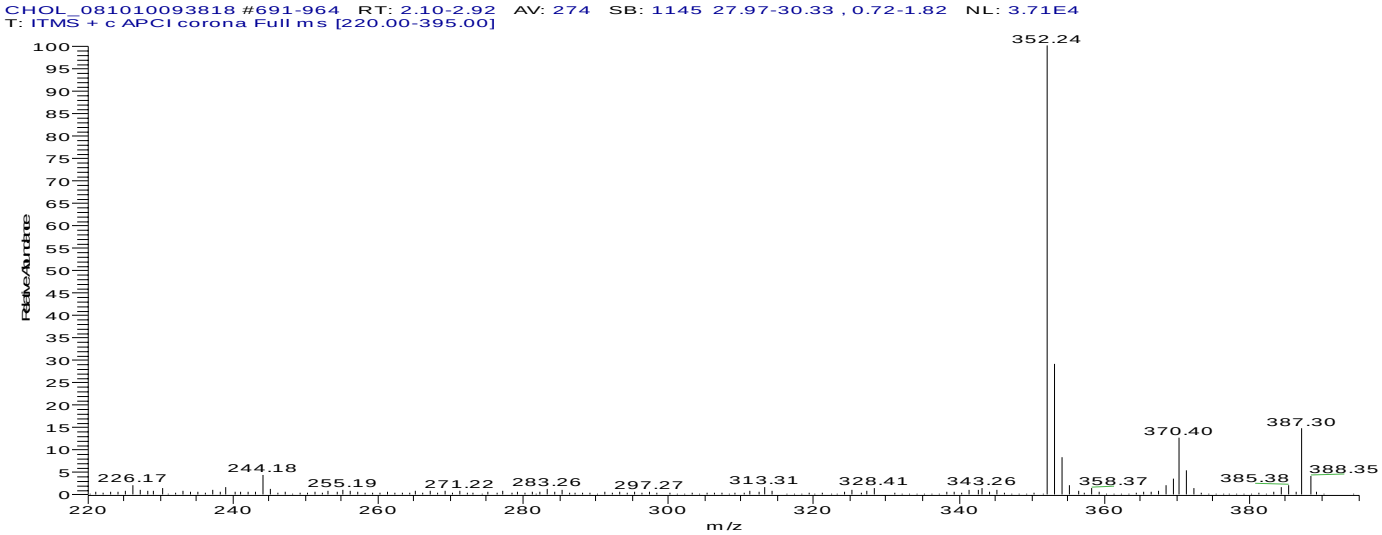

Figure S10. Mass Spectrum for peak at 2.7 min (cholesterol).

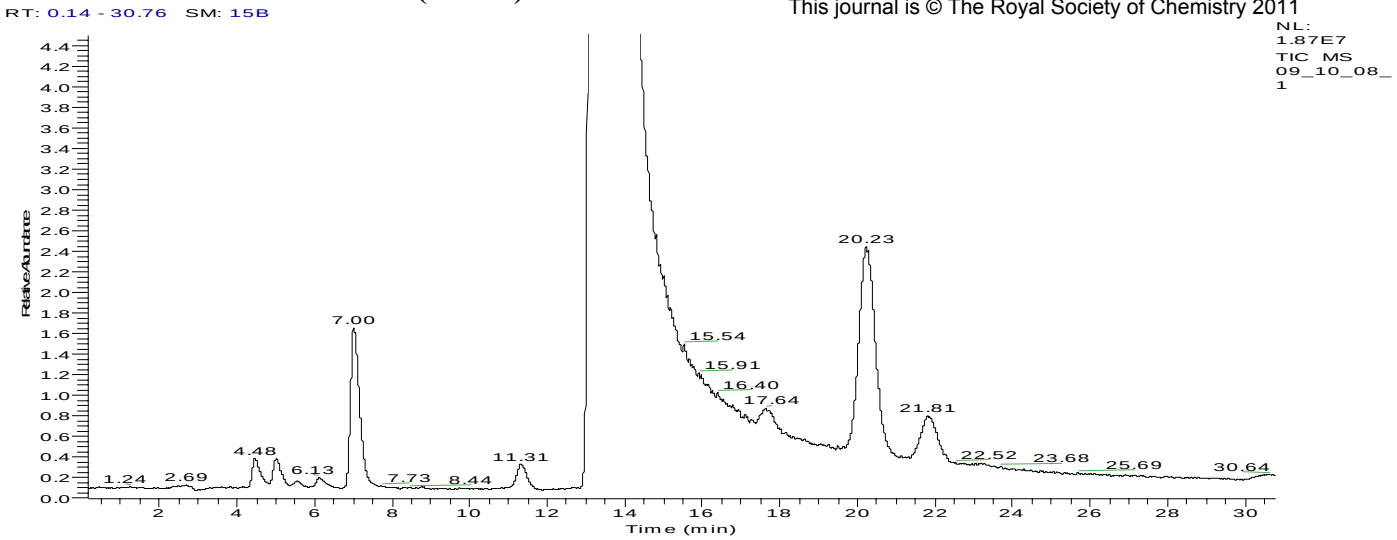

Figure S11. LC Trace for 4-PREGNEN-20α-OL-3-ONE.

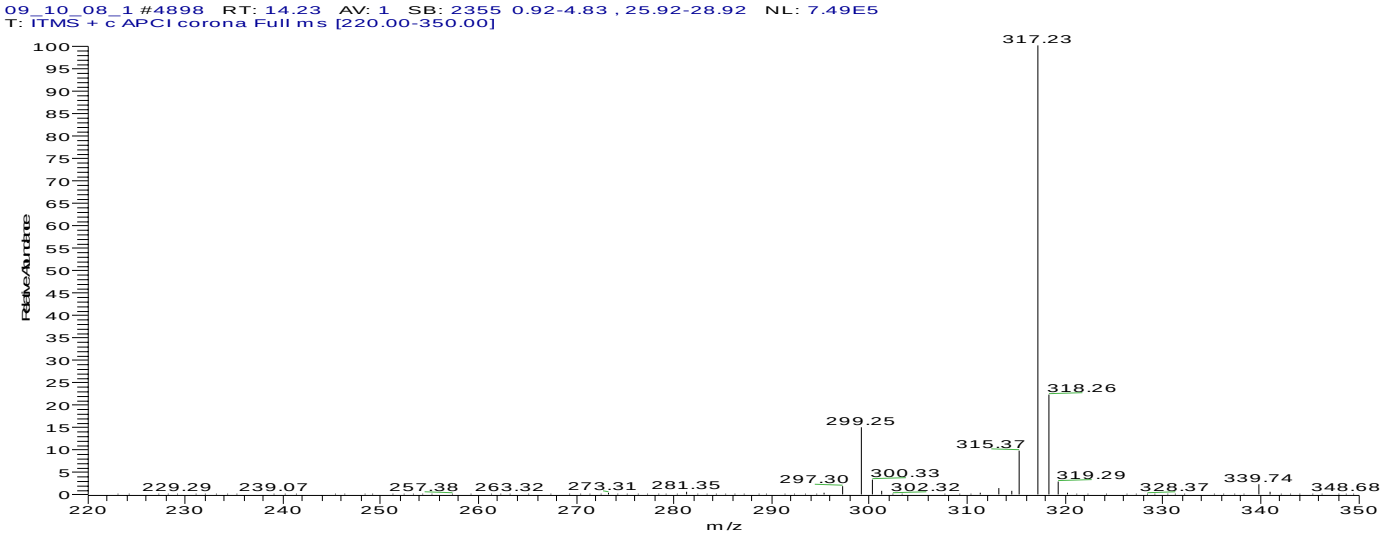

Figure S12. Mass Spectrum for peak at 13.53 min**.**

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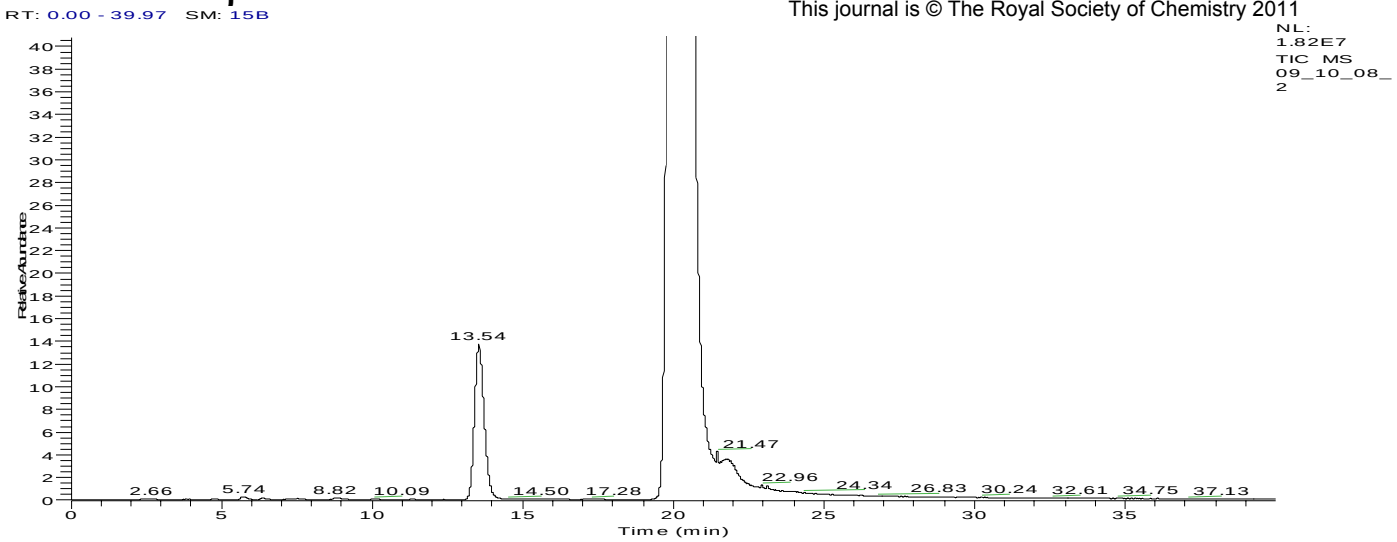

Figure S13. LC Trace for 4-PREGNEN-20β-OL-3-ONE. Major peak at 20.1 min is beta, and small amount of alpha present at 13.54 min

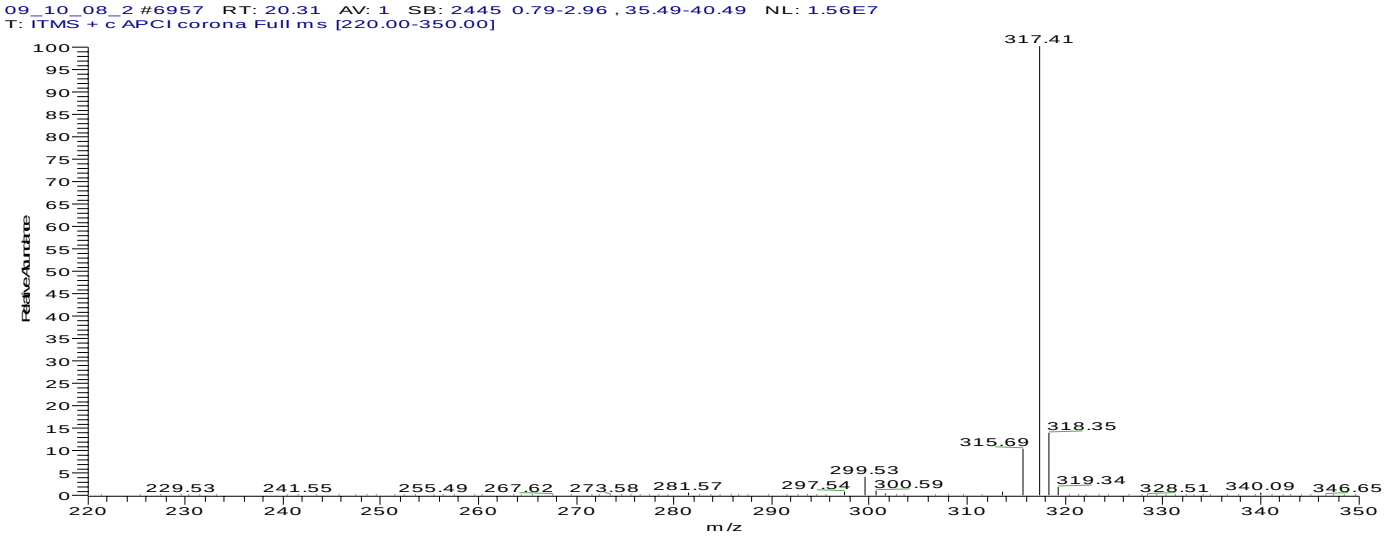

Figure S14. Mass Spectrum of major peak at 20.1 min.

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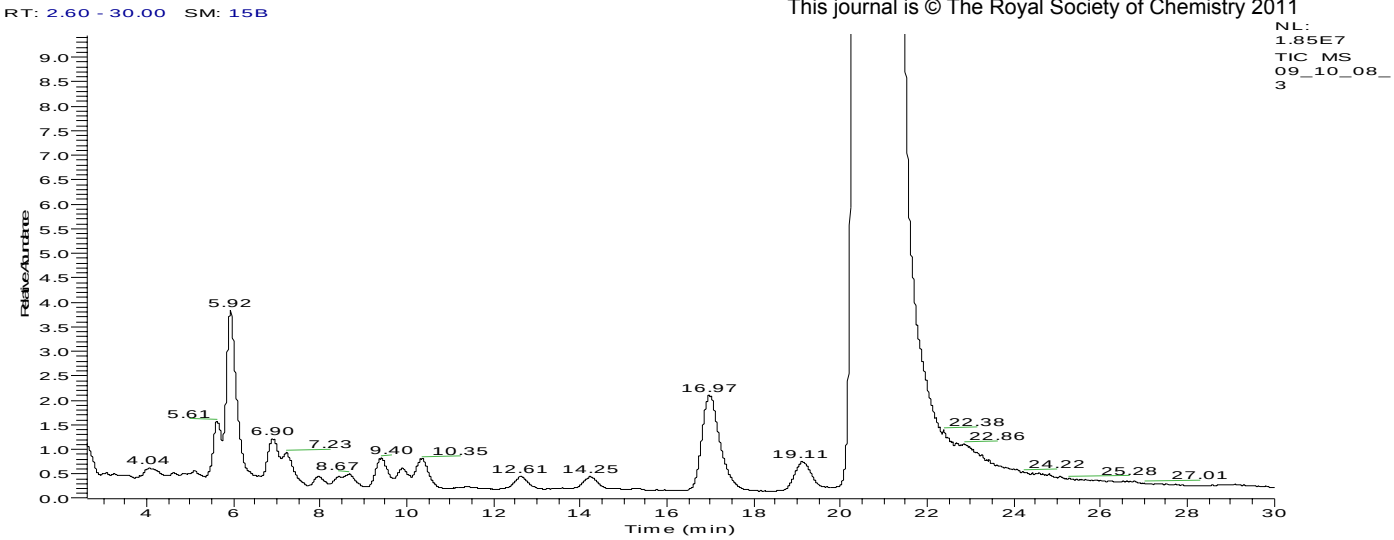

Figure S15. LC Trace for 16-DEHYDROPROGESTERONE.

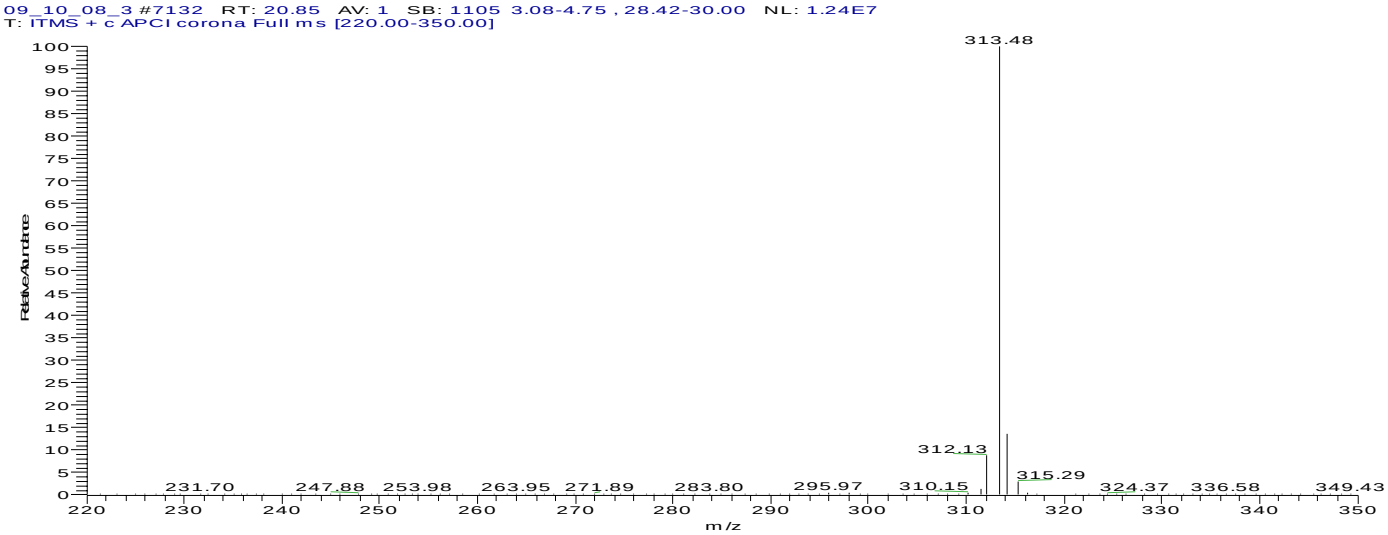

Figure S16. Mass Spectrum for major peak at 20.7 min.

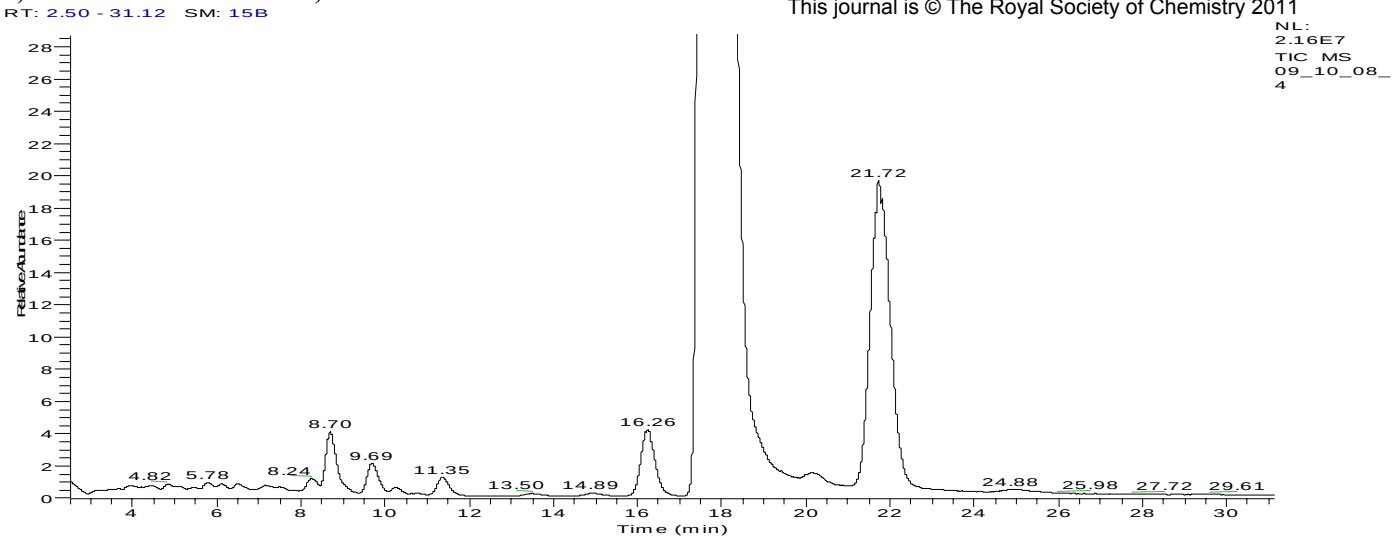

Figure S17. LC Trace for 4,6-PREGNADIENE-3,20-DIONE.

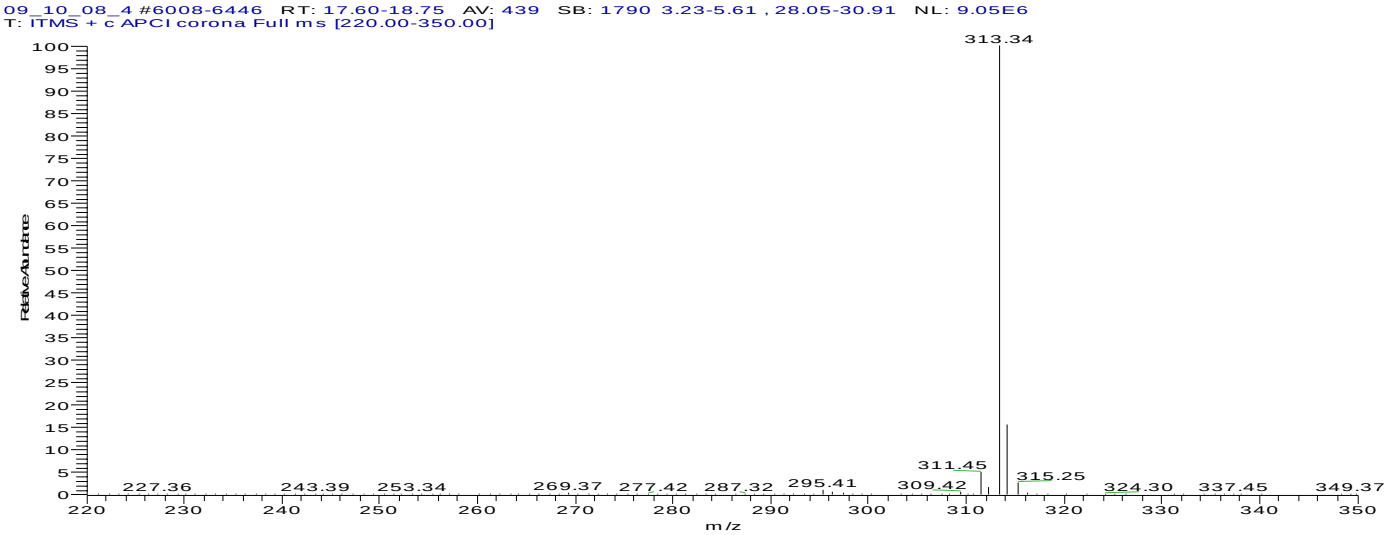

Figure S18. Mass Spectrum for peak at 17.7 min.

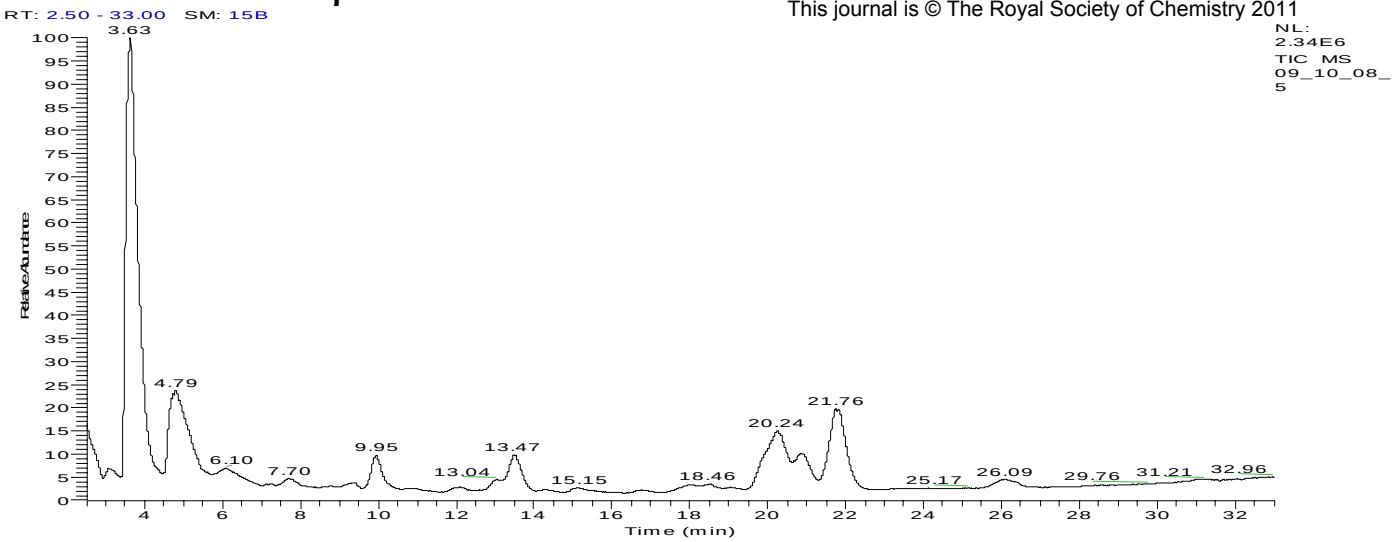

Figure S19. LC Trace for 4-PREGNEN-3-ONE-20β-CARBOXALDEHYDE.

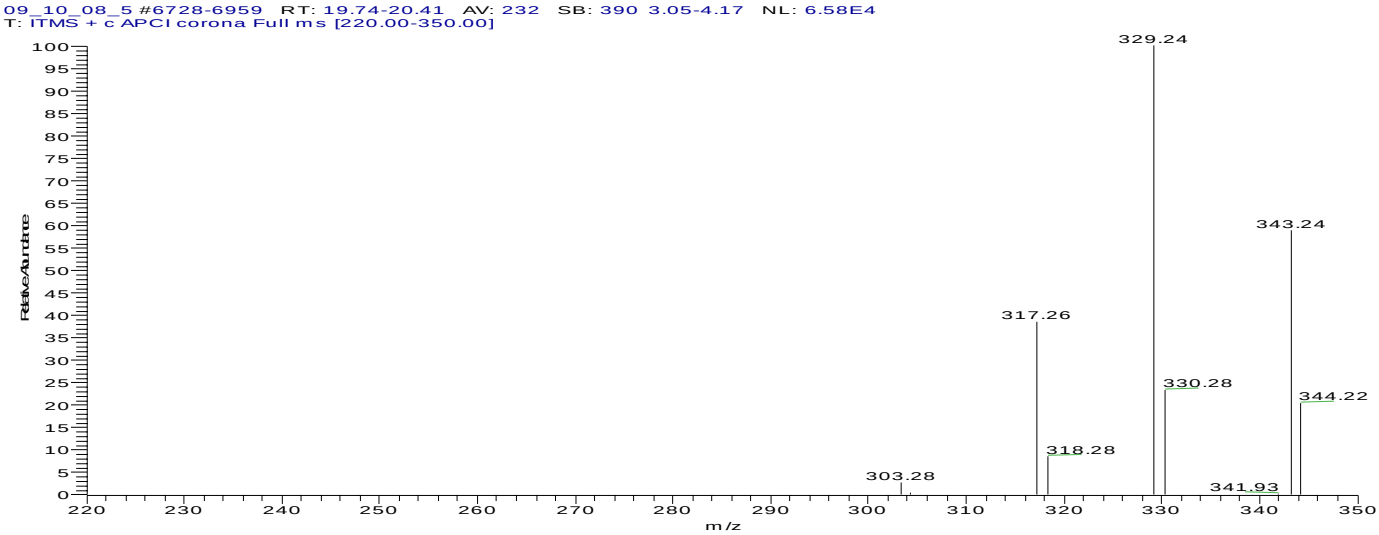

Figure S20. Mass Spectrum for peak at 20.24 min.

9. Carbon-13 NMR spectra of reference materials

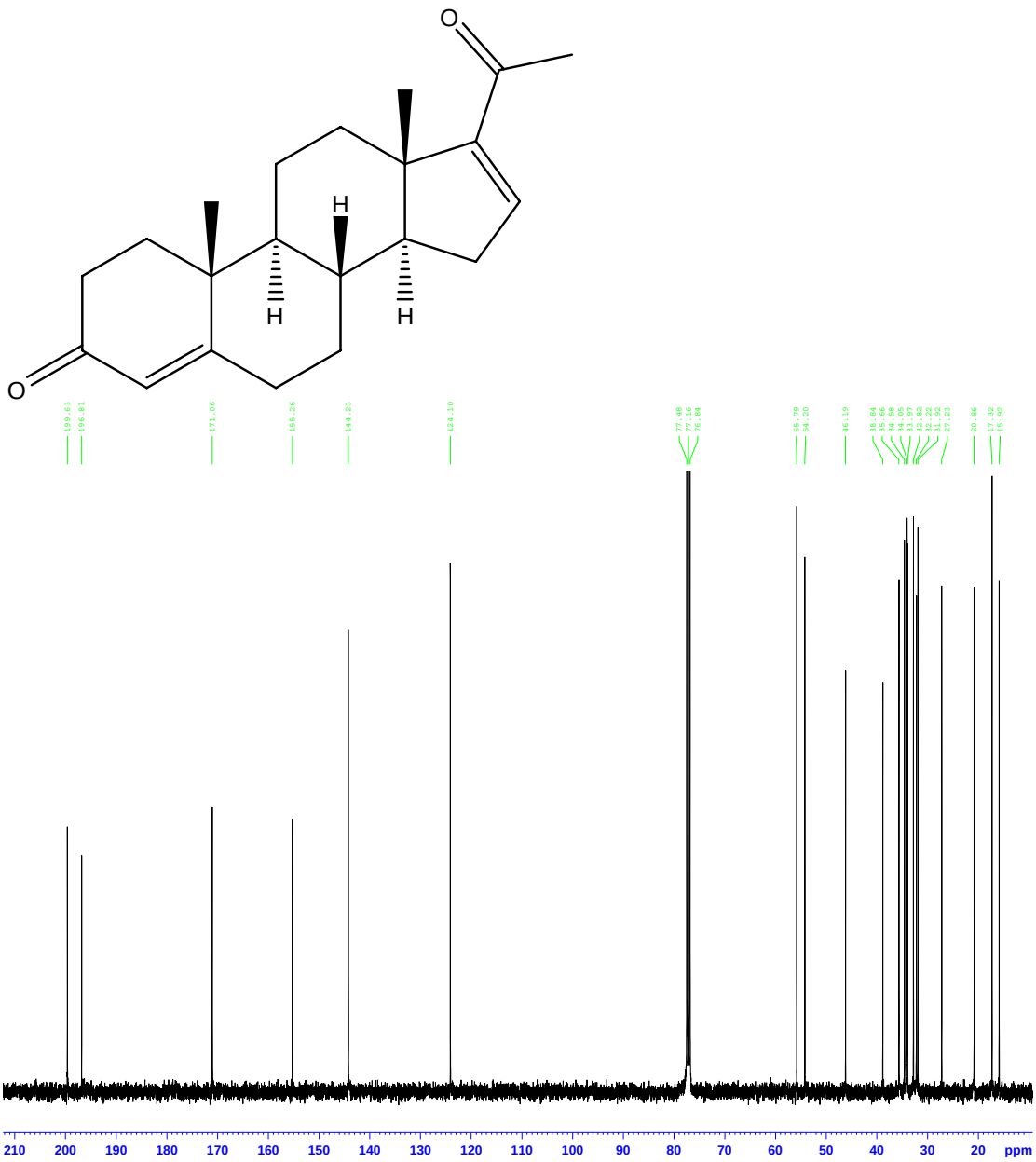

Figure S21. 16-dehydroprogesterone shows peaks at δ 199.6, 196.8, 171.1, 155.3, 144.2, 124.1, 55.8, 54.2, 46.2, 38.8, 35.7, 34.6, 34.1, 34.0, 32.8, 32.2, 31.9, 27.2, 20.9, 17.3, 15.9.

Figure S22. 4-Pregnen-20β-ol-03-one shows peaks at δ 199.6, 171.5, 123.8, 70.4, 58.3, 55.4, 53.8, 42.36, 39.6, 38.6, 35.7, 35.5, 34.0, 32.9, 32.1, 25.6, 24.4, 23.8, 20.9, 17.4, 12.4.

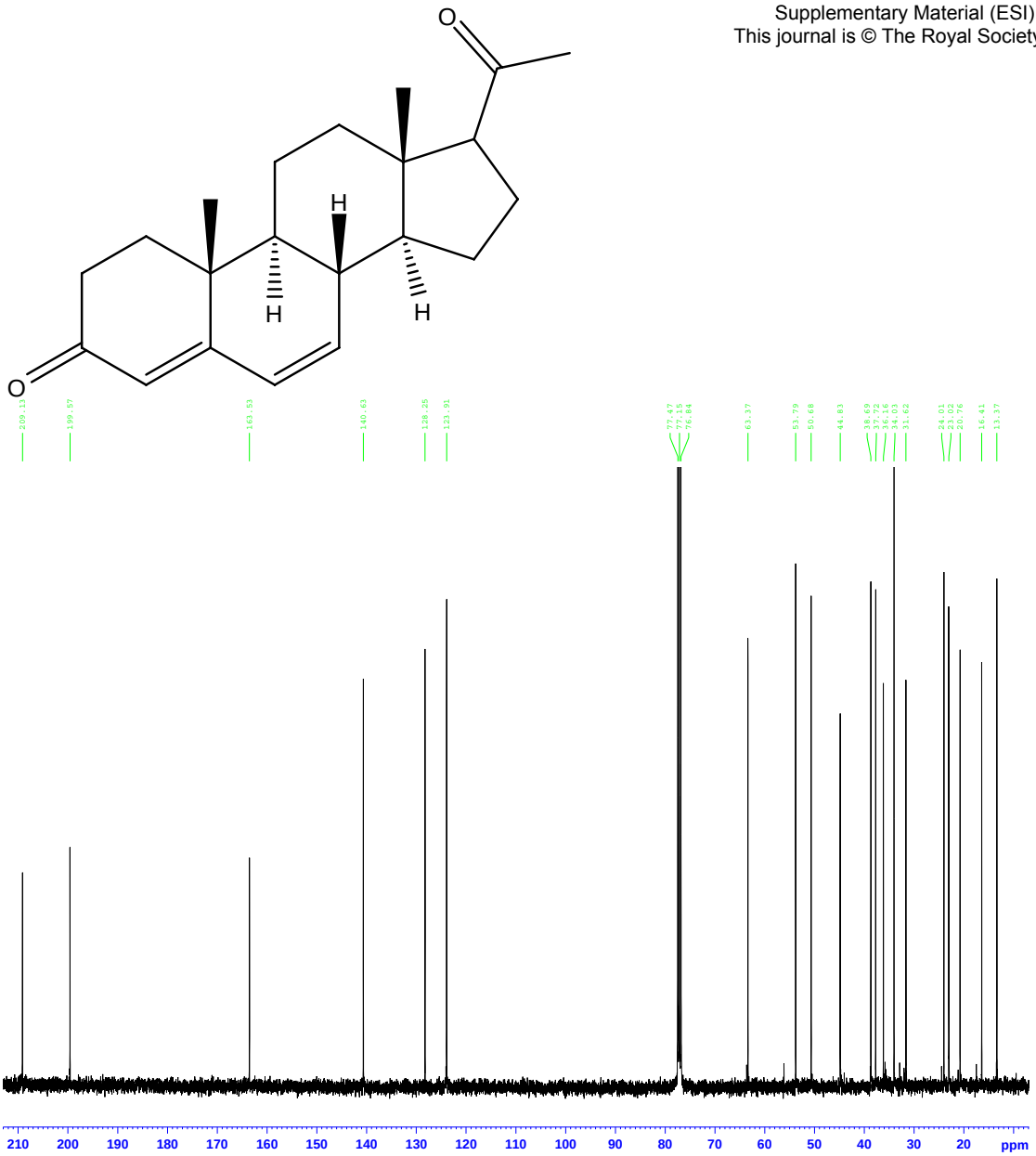

Figure S23. 4,6 Pregnadiene-3,20-di-one shows peaks at δ 209.1, 199.6, 163.5, 140.6, 128.3, 123.9, 63.4, 53.8, 50.7, 44.8, 38.7, 37.7, 36.2, 34.0*, 31.6, 24.0, 23.0, 20.8, 16.4, 13.4

* Intense peak, probably 2 peaks overlapping.

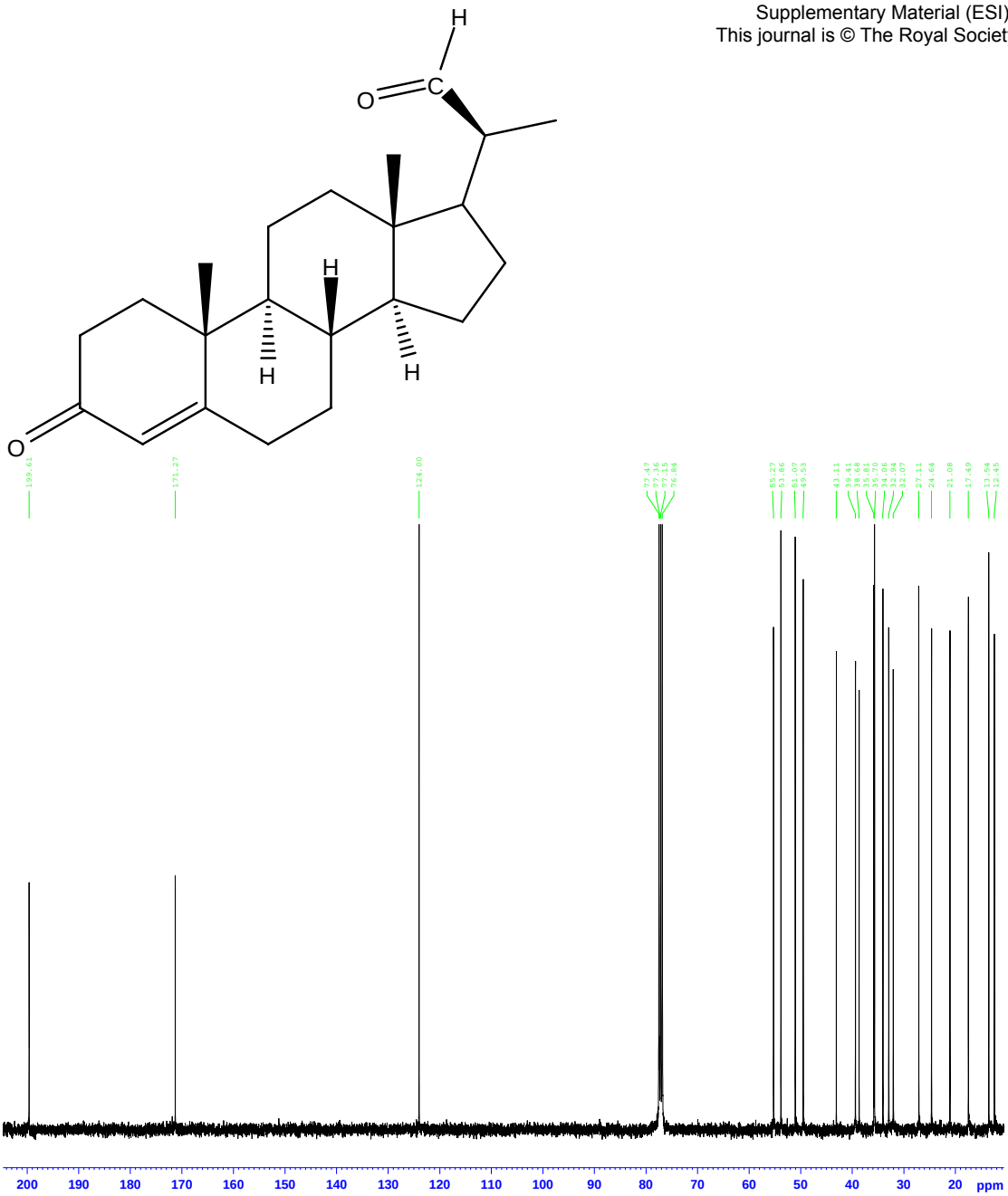

Figure S24. 4-Pregnen-3-one-20-β–carboxyaldehyde shows peaks at δ 205.0, 199.6, 171.3, 124.0, 55.3, 53.9, 51.1, 49.5, 43.1, 39.4, 38.7, 35.8, 35.7, 34.1, 32.9, 32.1, 27.1, 24.6, 21.1, 17.5, 13.5, 12.4.

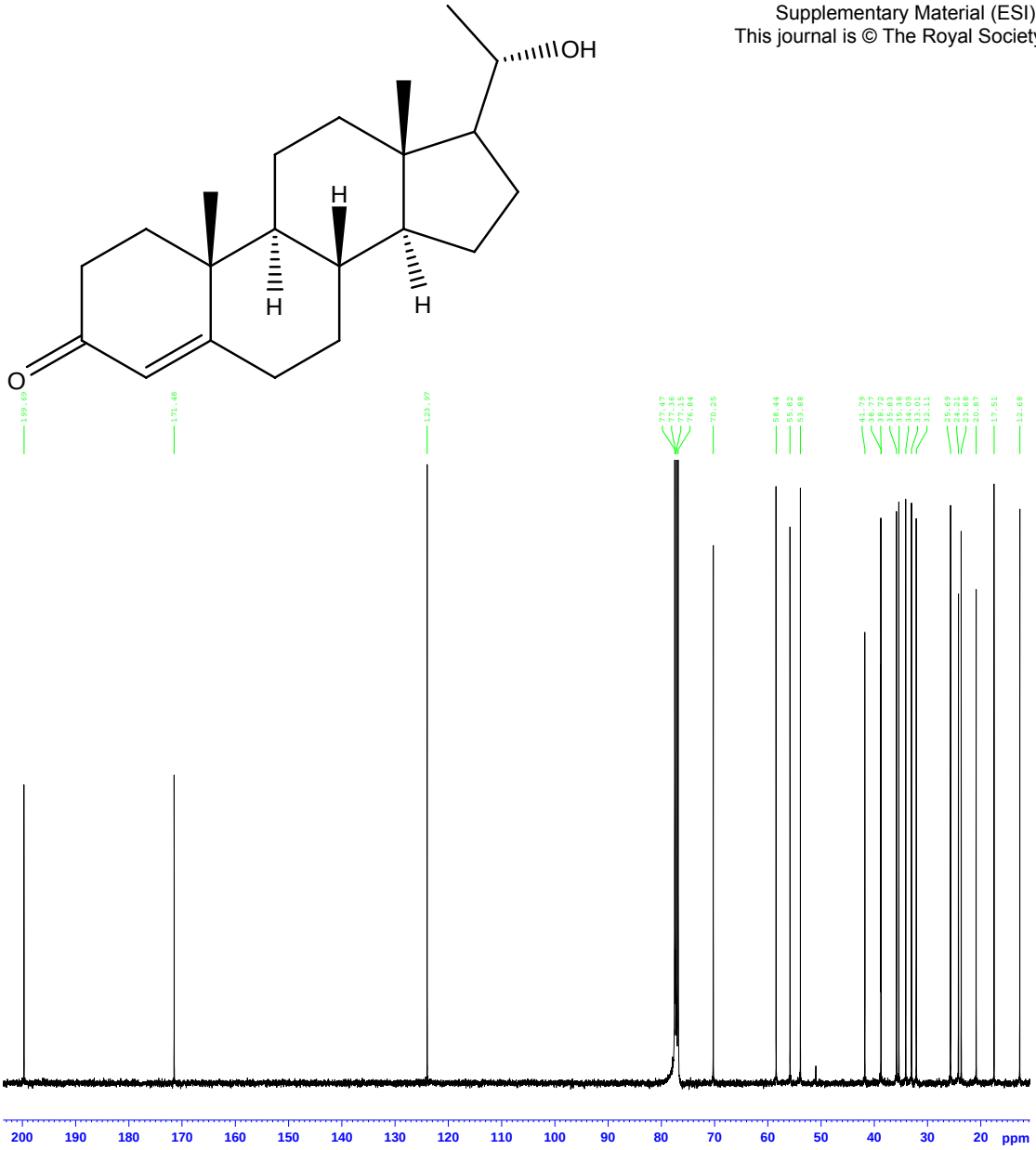

Figure S25. 4-Pregnen-20α-ol-3-one shows peaks at δ 199.8, 171.4, 124.0, 70.3, 58.5, 55.8, 53.9, 41.8, 38.8, 35.8, 35.4, 34.1, 33.0, 32.1, 25.7, 24.2, 23.7, 20.9, 17.5, 12.7.