Synthesis and analytical characterization of purpurogallin - a pharmacologically active constituent of oak galls

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ABSTRACT

Purpurogallin (PPG), an orange-red crystalline compound from nutgalls and oak bark, is an exemplar of numerous, ubiquitous natural colorants, biosynthesized through oxidative dimerization-decarboxylation of phenolic compounds. It possesses antioxidant, anticancer, and anti-inflammatory effects. Herein, a robust method is presented to allow students to expediently make this interesting compound that contains a tropolone ring, whose identity initiated a paradigm shift in the understanding of aromaticity. 1D and 2D nuclear magnetic resonance (NMR) spectroscopic data ^1H, ^{13}C DEPT-Q, HSQC, HMBC) were used to identify which protons are connected to carbon atoms, in contrast, to those that are exchangeable with D_2O. Fourier transform Infrared spectroscopy (FT-IR), mass spectrometry X-ray diffraction were used to provide complimentary information. Importantly, sufficient historical detail is provided to provide a useful narrative that highlights the shift from deductive and synthetic proof of structural identity to modern methods of structural elucidation. As a minor, but important aspect of iron gall inks, PPG links the work of important western historical documents from Da Vinci's Vitruvian Man to the Declaration of American independence. In the experience of the authors, such a pedagogic approach enlivens students with practical exercises which place them within the historical arc and reasoning of notable scientists, adding a sense of scientific discovery.

KEYWORDS

Graduate Education/Research, Hands-On Learning / Manipulatives, Organic chemistry, Synthesis, Natural Products, NMR spectroscopy

INTRODUCTION

The history of science provides a resource from which to learn the thought processes dictating the previous steps of scientific inquiry. It is common for teachers to draw on the history of chemistry[1] as a way to engage their students and state scientific facts. Many studies have confirmed the efficacy of the historical approach in chemistry teaching and especially its importance for the qualitative understanding of concepts.[2-6] The purpose of this laboratory exercise is to provide students with organic synthetic chemistry and structural elucidation skills, set alongside historic imaginative problems. Here, an organic synthetic chemistry practical is placed in context of aromaticity and the development of inks using galls.

In 1942, the noted natural product chemist Harold Raistrick, a protégé of F. Gowland Hopkins[7], together with his coworkers, detailed the isolation of the fungal metabolite stipitatic acid **1** ($C_8H_6O_5$) from *Penicillium stipitatum* (Figure 1).[8,9] Michael J. S. Dewar,[10] then working with Sir Robert Robinson[11] was drawn to their work and correctly deduced that Raistrick's results pointed to a hitherto unrecognized non-benzenoid aromatic system.[12]

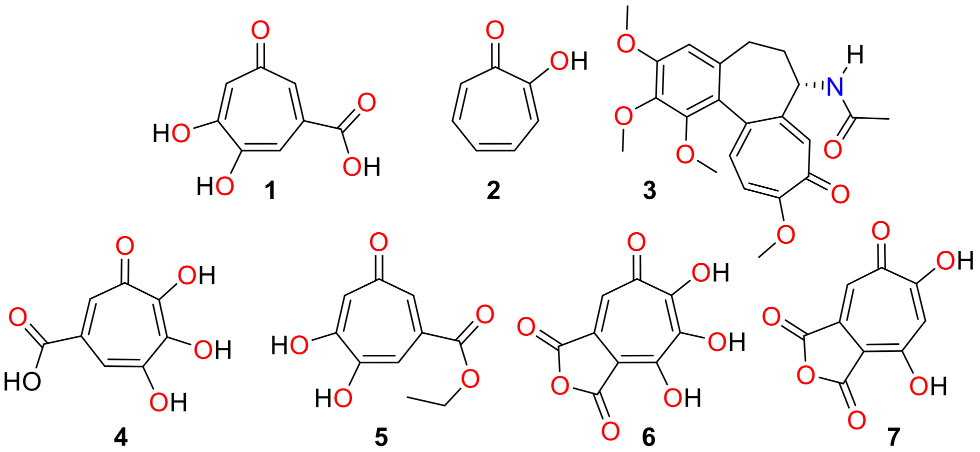
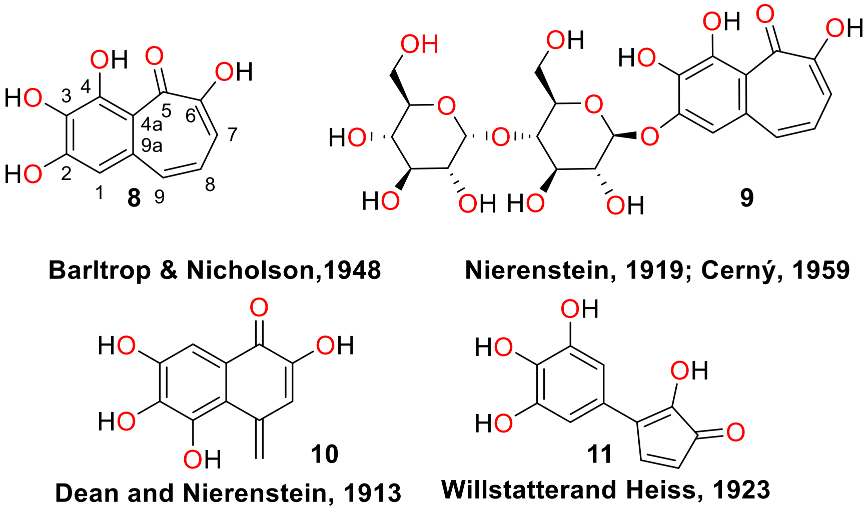


Figure 1. Various tropolones. **1**: Stipitatic acid; **2**: 2-Hydroxy-tropolone; **3**: Colchicine; **4**: Puberulic acid; **5**: Ethyl stipitate; **6**: Puberulonic acid; **7**: Stipitatonic acid; all of which except **2** are found naturally.

Dewar named this seven-membered ring system tropolone **2** (2-hydroxycyclohepta-2,4,6-trien-1-one) and shortly thereafter, he also postulated a structural revision of the natural product colchicine **3** (ex. *Colchicum autuminale*).[13] This quickly led to solving the structures of all five mould tropolones **1**,**4**-**7**, as well as other natural products possessing the tropolone ring. Additionally, working at Oxford was the talented chemist John A. Baltrope, who also did his initial work under the tutelage of Sir Robert Robinson. He was interested in the structure of purpurogallin **8** ($C_{11}H_8O_5$; Figure 2), a synthetic analogue[14] of natural red coloring matter dryophantin **9** known to exist in various galls.[15,16,17] These "oak apples" are produced by various plants during wasp infestation and egg-laying and can be found in a variety of shapes and colors. After a detailed chemical investigation, including synthesis and chemical transformations, and also referring to the ideas of Dewar, Baltrope and Nicholson, revised the previously postulated structures **10** and **11** to a troplone **8** a hydrolysis product of dryophantin (Figure 2, **9**).[18]



8 Barltrop & Nicholson,1948

9 Nierenstein, 1919; Cerný, 1959

10 Dean and Nierenstein, 1913

11 Willstatterand Heiss, 1923

Figure 2. Purpurogallin **8** and its precursor dryophantin **9.** The two structures postulated to be **8** were **10**: 2,5,6,7-tetrahydroxy-4-methylenenaphthalen-1(4H)-one and **11**: 2-hydroxy-3-(3,4,5-trihydroxyphenyl)cyclopenta-2,4-dien-1-one. Although much of Baltrope's work was never published,[15, 16] he suggested that **8** could demonstrate anticancer properties and with subsequent investigations it and similar molecules possess such properties.[17,18] Note that Nierenstein[19] published a method for isolating dryophantin **9** in 1919, it was not until 40 years later that the structure was confirmed by Černý.[20]

As a molecule **8**, also constitutes a minor but important component of iron gall inks that have been used to write some of the most important western historical documents including: The Domesday Book, the Charter of Liberties (i.e. The Magna Carta), Leonardo da Vinci's notebooks, The American Declaration of Independence, and many others.[21-22] Gallic acid produced from fermentation of oak galls produces a pure black colour ink used on these papers and today in fountain pens. Before a fermentation step with a mold, oak galls release other tannins such as gallotannic acid which produces a darker brown pigment.[23] Ironically, in some cases, such inks have eroded the material on which they have been written.[24] Research towards the preservation and understanding of old manuscripts has necessitated the development of non-destructive spectroscopic imaging.[25]

In other areas, the use of oak galls has predated this work right back to the 1300s, when ground, powdered gall nuts were used to test the iron concentration in spa waters. Physicians at the time believed the iron ions present in such waters were vital as a therapy for different conditions with the source of the therapy to be spa water. Different depictions of the test have been noted throughout history but most credited was the chemist Sir Robert Boyle (1627-1691) who quantified the method. The iron (II) ions react with the gall nut producing a colour change test – a deep purple solution.[26]

Polyphenols are now a rich class of compounds, particularly having been identified as phytochemicals. For example, the theaflavins from the leaves of *Camellia sinensis*, have a tropolone ring that originated from the oxidation of polyphenols.[27]

In this paper, we present an expeditious and robust synthesis of **8** suitable as a laboratory exercise for undergraduates and postgraduates, which can be adapted for high school level. Scripts for different ages are supplied in the Supporting Information. Previously, van Opstal *et al.* have described the use of oak gall extract to quantitatively determine the amount of iron present in a water sample using a historically contextualized laboratory for undergraduates.[26] Also, Martin-Gil *et al.* have placed galls in historical context in a practical experiment for undergraduates studying the stability of fountain pen ink.[28]

EXPERIMENTAL OVERVIEW

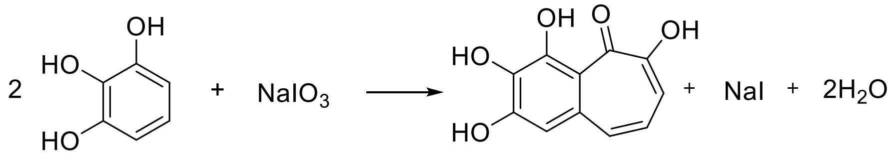
The experiment for undergraduates is designed for an introductory, apparently “simple”, exercise in structural determination utilizing Fourier transform infrared spectroscopy (FT-IR) spectroscopy, mass spectrometry (MS), one and two-dimensional nuclear magnetic resonance (NMR) spectroscopy and the use of X-ray diffraction data, for students who already have some knowledge of solving structures using a variety of spectroscopic and spectrometric data. It specifically presents the synthesis in the historical context of early pioneers of natural product synthesis which highlights the methods which were originally used to ascribe structures before the widespread adoption of powerful structural determination methods such as magnetic resonance (i.e. spectroscopic) and electrospray methods (spectrometric methods) signaling a paradigm shift in structural elucidation methodology.[29] The experiment works as a guided inquiry experiment where the students are given an academic paper and they devise the protocol. The experiment has run over 10 years with a class of around 15 undergraduate or MSc students. The learning outcomes are as follows:

1. Laboratory procedure derivation and basic synthetic skills.
2. Analysis using NMR, FT-IR, MS, and X-ray diffraction.
3. Structural determination and comparison of methods.

Numerous preparations have been reported for the synthesis of **8** with varying quality. “Purpurogallin” from one commercial vendor (Aldrich) was similar to the crude, brown, material synthesized by the method reported here, whereas, our purified material is orange, consistent with the work of Scharpenseel.[30] This is a definitive indicator for an instructor seeking to determine whether students have completed the purification steps with competence. Having investigated various methods to ascertain reproducibility for a previous industrial study that required 5 kg of **8**, as an advanced pharmaceutical intermediate, in our hands, the method of Evans and Dehn[14] was found to be the most convenient, expedient and, importantly, reproducible. The experiment requires neither sophisticated apparatus nor complex regents. It can be used as a synthetic exercise which includes a dramatic production of color (see Video#1, Supporting Information) and can be coupled with a simple melting point determination and/or TLC.

Before a pre-lab class, students were asked to read the undergraduate handout (Supporting information) which poses the question: *Where do we get ink from?* The students are also asked to download and read the paper by Evans and Dehn[31] and are asked to design the experiment themselves, including a suitable scale, list of equipment, required chemicals, and an environmental assessment of any waste generated. During the pre-lab classroom session, the students are encouraged to share their thoughts on the history of inks followed by discussion of the experimental procedure. They are asked by the instructors if the information they have provided is sufficiently detailed to replicate the preparation. In line with modern practice, students fill a safety evaluation (COSHH form) and identify any hazards/toxicity and plan to take suitable precautions including lab coat, safety glasses and/or visor and nitrile gloves.

The experimental synthetic procedure is fully explained in the Notes for Instructors in the Supporting Information. The experiment consists of the oxidation of pyrogallol by sodium iodate (Equation 1).



(Equation 1)

The students add the oxidant slowly i.e. drop-by-drop from a dropping funnel under rapid magnetic stirring, causing the triol to oxidize and self-dimerise. The unstable compound then undergoes a complex series of tautomerizations and also expels a gas (CO_2), and forms both sodium iodide and water. Students should note a red brown precipitate (see Supporting Information, Videos#2&#3). They are then asked to filter off the precipitate under vacuum, wash and dry to form a solid cake. Students were then instructed to dissolve the filter cake in boiling 95% per ethanol/distilled water (v/v). This solution was filtered and then precipitated with 2.5 volumes of distilled water. Students were advised to take further purification steps with precipitation from ethanol. The average yield was 78% (±5) and high-resolution mass spectrometry data was obtained: (ESI^+) Calcd. for $C_{11}H_9O_5$ ($[M+H]^+$), 221.0445; found, 221.0442), which corresponded to literature values.[32]

In an additional lab, students were asked to prepare and perform a series of different analytical procedures on their samples to confirm their own synthetic accuracy, reproducibility of the method and structural identity of the compound. These included thin layer chromatography (TLC), FT-IR, mass spectrometry and several types of NMR.

The full experimental procedure was advised to be conducted in two, three-hour lab classes including the analysis segment. For analysis lab, it was preferred that the labs were conducted on the same day, so samples were fresh for analysis. The students were encouraged to work independently or in pairs. One instructor per fifteen students (or as school policy follows). Students were advised to carry out mass spec on their own as sufficient training would be given earlier in the semester, however, a technician was on-hand to help supervise when needed.

All students were expected to record their observations in a hardback book. All data was exported from instruments such as infra-red in ASCII format and plotted in graphical software. The students were also expected to produce a written report that utilised a professional template from a major scientific publisher such as American Chemical Society. This type of format allowed the student to place artwork and tables close to the point where they were discussed in the text. This ensureed that the students were prepared for a future career either in academia, further study or industry. The template facilitated the marking process and standardises the work. Submission was anonymous and electronically. Marking used an electronic platform and rubric: the normal format used no more than four pages and correspondedto a typical communication.

The following marking scheme has been successfully used:

Abstract 10%:

Introduction 20%

Materials and methods 5%

Results and discussion 40%

Figures 15%

Conclusion 5%

References 10%

Production 5%

The written report counted 40% towards the module and the remainder was by written examination where one question was on structural determination. Although we tried a peer review process by allowing students to mark one another this has never proved consistent and consequently marking was completed by one educator alone since the cohort has been sufficiently small. A consistent problem has been variation in the quality of references and students are advised not to rely on internet sites and all papers must be original. The use of a referencing program was encouraged.

HAZARDS

On the basis of the available animal and clinical data pyrogallol is safe as a cosmetic ingredient in the present practices of use and concentration.[33] This compound has a good safety profile [Pyrogallol LD_{50} = 300 mg/kg (Mouse)][34] but can be corrosive to the skin or when inhaled at high concentrations. Sodium iodate is considered toxic. It should be noted that younger children may be less careful in handling the oxidant. With trials of different solvents for a “greener” method, we noted a small explosion through simple dry grinding and mixing of pyrogallol with sodium periodate in a mortar and pestle. Consequently, we strongly discourage everyone to avoid the mechanochemical grinding variant of these experiments. No problems have ever been encountered when solutions are slowly mixed and there is only a minor exotherm (up to 30 ˚C). The oxidant is always added to the pyrogallol to maintain an excess of reducing agent. Inverting the addition is not dangerous but only produces a very small amount of purpurogallin dissolved within a black tar which cannot be extracted.

Depending on the age of the participant, the synthetic protocol is slightly modified in terms of scale. However, all students (including children) have to wear gloves, refrain from wearing contact lenses and wear safety glasses and be accompanied with a lecturer/demonstrator. Experiments are normally carried out in a fume hood but demonstrations have been carried out on the bench provided solutions are dosed either with dropping funnel or a titration burette for smaller scales.

RESULTS AND DISCUSSION

This experiment was performed as an introduction to the concept of synthesizing natural products from simple precursors that self-dimerize and expel gas. In laboratory, the students were able to set-up the experimental apparatus and mix the reagents with little help from the instructors. During the experiment it is not obvious that it is a dimerization and the visual change provides a dramatic illustration of reaction progress. The students are asked to carefully record all their observations and some failed to observe the evolution of gas; as Pasteur noted in his inaugural lecture to The faculty of sciences at Lille (France, 1854); “In the field of observation, chance favors only the prepared mind” [La chance ne sourit qu'aux esprits bien préparés]. The released gas can be trapped and reacted with limewater to demonstrate that it is carbon dioxide. Overall, the pre-class appeared to work to make the students decide on the correct course of actions in most instances. This was supported by student survey results that showed a majority of students felt that being allowed to design the experimental protocol had positively impacted their learning.

Comparison of the student ^{1}H NMR spectra (Figures 4) reveals experimental consistency between students and similar impurity profiles. The students were successful in realizing that not all molecules can be identified from ^{1}H spectra alone and they were encouraged to examine the 2D (DEPT)-Q experiments to determine the multiplicity of each peak and correlations, which allowed them to deduce it was some form of unsymmetrical dimerization and with some prompting, that the loss of CO_2 would produce an unsymmetrical compound. Figure 3 illustrates our approach of first working out the multiplicity using Distortionless Enhancement by Polarization Transfer (DEPT)-Q and then linking to the ^{13}C to ^{1}H nuclei through the use of heteronuclear multiple bond correlation (HMBC) and heteronuclear single quantum coherence (HSQC). This was then followed by cross examination with the IR and mass spectrometry data.

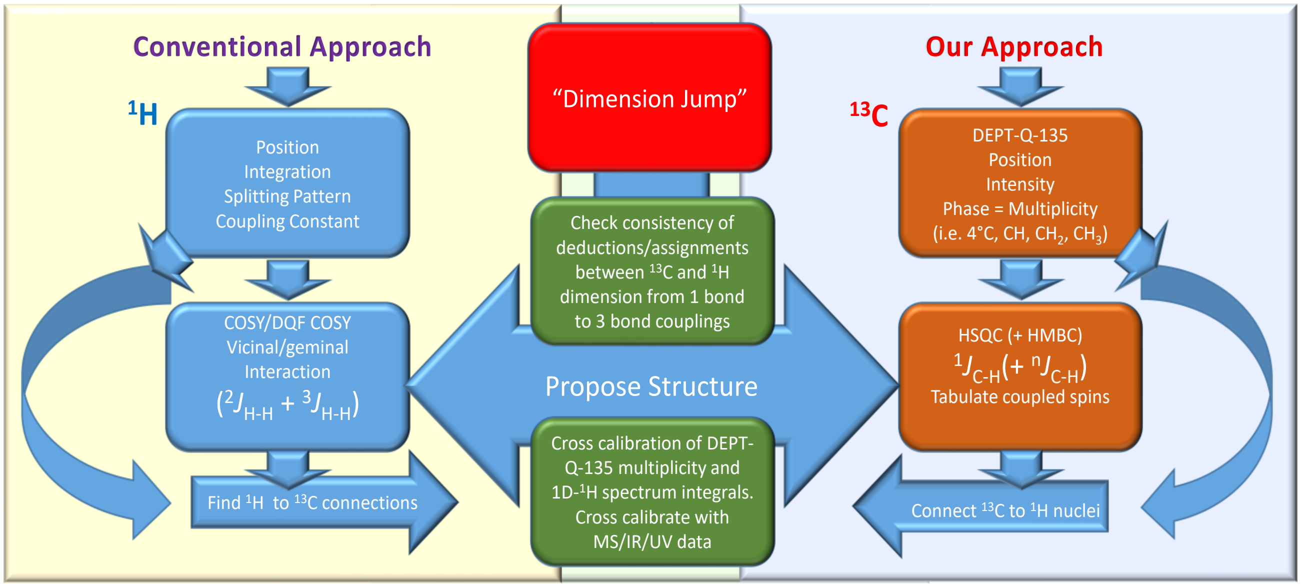


Figure 3. Diagram of different NMR approaches used by students to solve structure. A full set of NMR spectra are found in the Supporting Information (Figures S1-13).

The DEPT-Q and HSQC data allowed clear determination of four methine carbons (Figure 5), which the students were able to appreciate as building blocks of certainty to build up a structure. This approach was developed in part from past post-lecture surveys that showed for students either attempting to initially assign and deduce the structure using ^{1}H NMR spectra they were faced with the difficulty of resonance overlap from a relatively narrow 0-10 ppm diamagnetic chemical shift range. A further obstacle to analysis was the ^{1}H homo-nuclear coupling present in the multiplets, which the students found difficult to understand, despite being taught the n+1 rule. Consequently, those new to spectroscopic analysis preferred to interrogate ^{1}H multiplets using ^{1}H by using DEPT-Q, cross-correlating each peak through HSQC experiments and using these to interrogate proton spectroscopy, and then confirming assignments using integral values of each assigned proton. Students who attempted structural elucidation were more satisfied with this teaching approach using ^{13}C NMR first because it reduced cognitive load.

The students had little difficulty in correlating all protons to the respective C-1, C-7, C-8 & C-9 protons using the DEPTQ ^{13}C spectrum. In this instance, the use of ChemDraw was encouraged to predict both the ^{13}C and ^{1}H spectrum of which the former closely resembled the shift positions found in DMS0-d_6 (see Supporting Information). They all concluded that HMBC NMR data as the most useful of the spectra available to them because they themselves could deduce connectivity patterns. Since INADEQUATE spectroscopy is taught[35] within the student courses as the closest alternative to X-ray crystallography, where they can directly trace out the carbon skeleton by NMR in solution, their request to acquire this data was noted but denied as HMBC was considered sufficient to solve the problem at hand.

Students had to deduce from the 2D NMR that the molecule contains three consecutive vicinal linked methines; that a singlet resonance in the ^{1}H NMR shows that the rest of that ring does not contain methines groups and must, therefore, be phenolic. The interpretation of the ^{1}H NMR spectrum requires a minimal number of assumptions. Generally, in protic solvents the -OH groups appear at room temperature as broad signals due to fast, on the NMR time scale, exchange of the OH protons with protons of the solvents.[36,37] If it is assumed that the proton situated at position 7 (adjacent to the hydroxyl group within the tropolone ring) will be the more deshielded of the two doublets (predicted from the (n+1) rule) it will resonate downfield of the only pair of doublets present. In this case at this concentration, it is found around 7.3 ppm. The correlated spectroscopy (COSY) spectrum served to support the assignments of illustrating ^{3}J H-H (vicinal) interactions within the tropolone ring (Figure 6). Consequently, the students were able to assign each of the protons in the spin system detected for the protons attached to carbons. The assignment of individual HO peaks was less obvious but the presence of a strong hydrogen bond at 15.3 ppm was assigned by most students since it is known to resonate at a position typical of that found in molecules containing a structural motif commonly found in flavonoids and related compounds. For MSc/PhD students, an incredible natural abundance double quantum transfer experiment (INADEQUATE) spectrum was also provided that allowed them to readily identify the presence of both a benzenoid and tropolone ring and deduce their points of ring fusion.

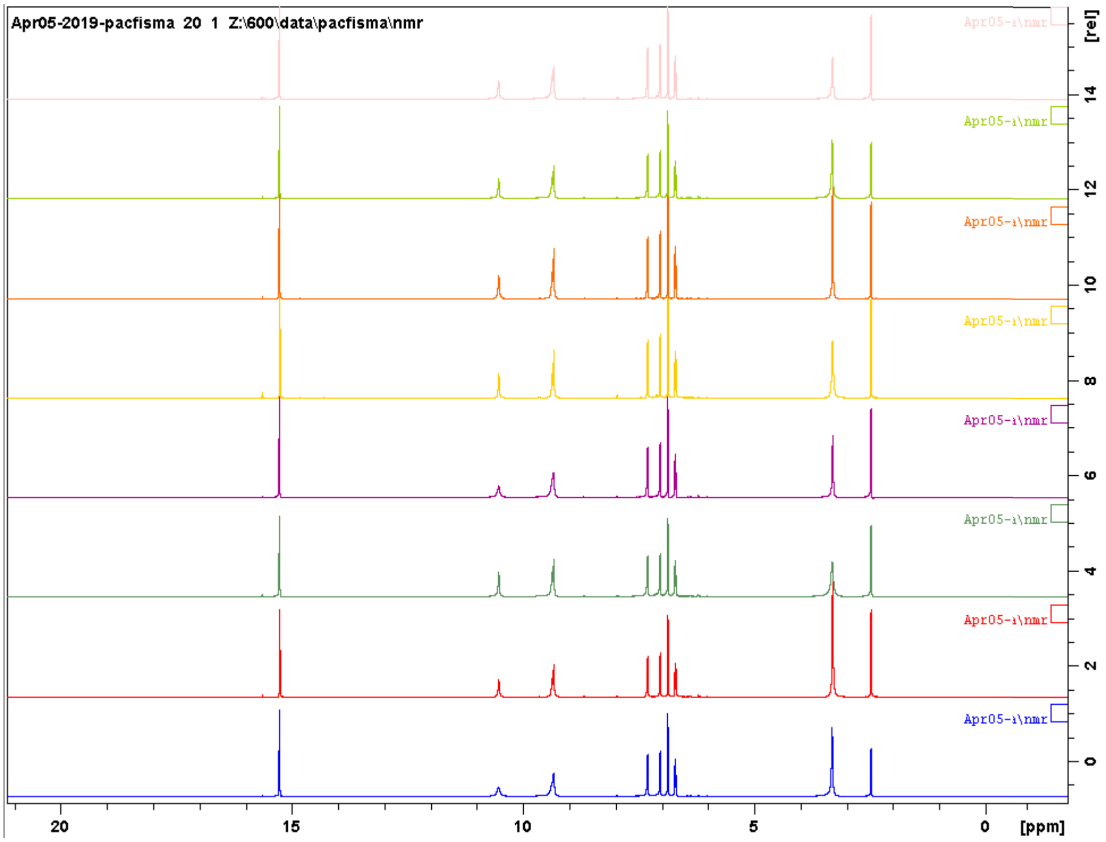


Figure 4. ^{1}H NMR (600 MHz, DMSO-d$_6$) spectra of purpurogallin **8** illustrated one above the other, for eight individual students demonstrating the robust nature of the synthesis. Note shift of internally hydrogen bonding phenol-carbonyl interactions resonating downfield. A comparison can be made to quercetin (See Figure S5). All spectra were acquired with deuterium locked automation sequences on the auto-sampler and have not been manually shimmed. Larger versions can be found in Figure S8.

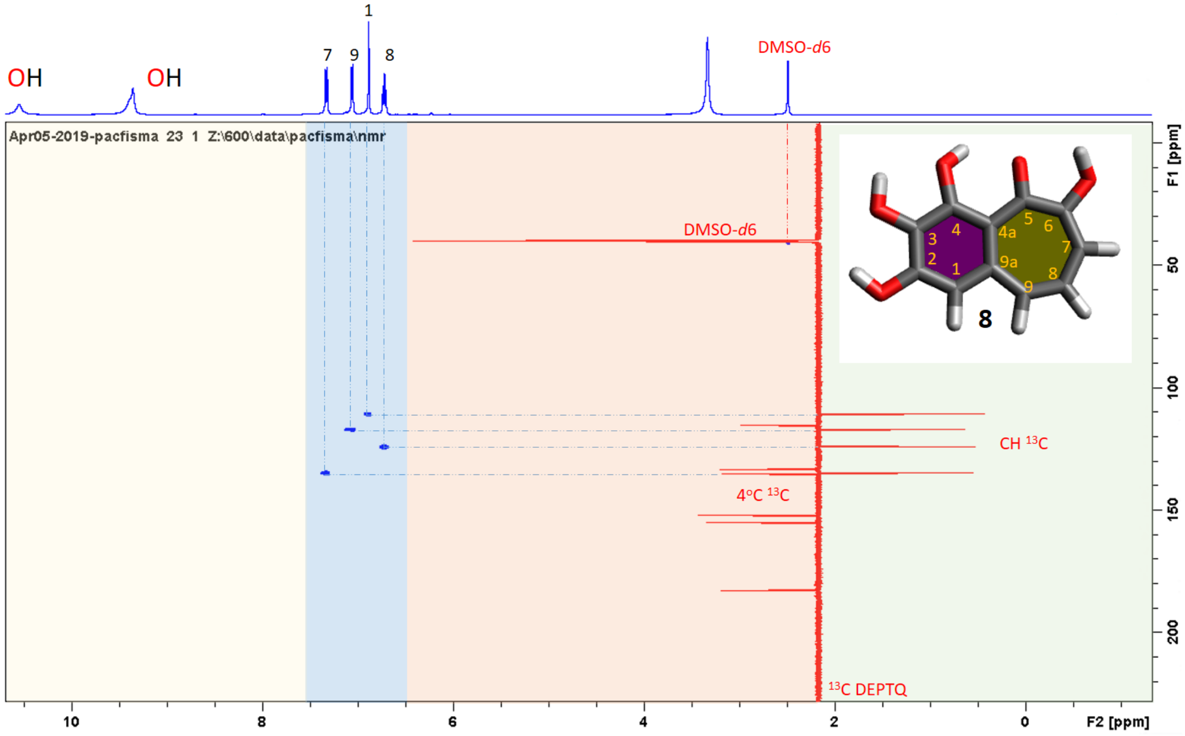


Figure 5. HSQC (600 MHz, DMSO-d_6) spectrum of **8** (Blue cross peaks) illustrating connection between methine carbons and the corresponding protons. The DEPT-Q experiment is superimposed to allow assignment of the multiplicity of the cross peaks. A larger version can be found in Figure S11.

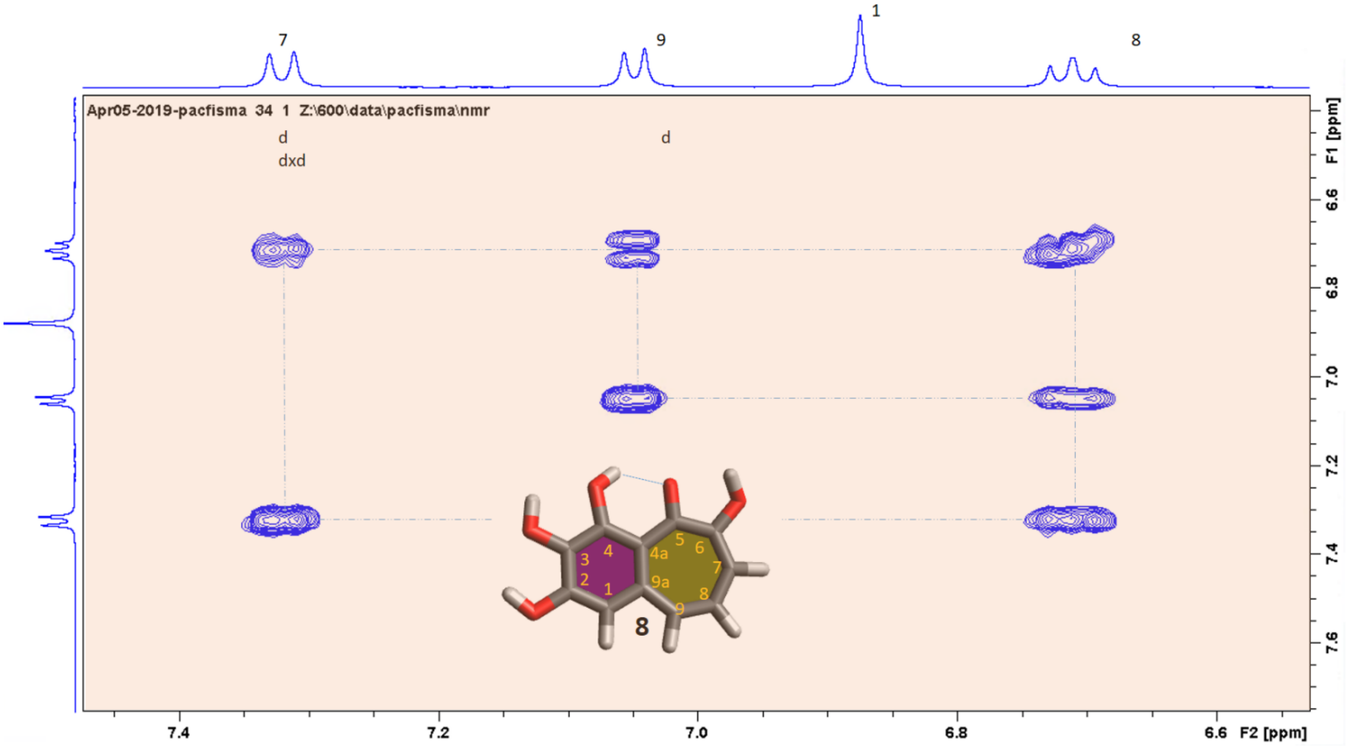


Figure 6. ^{1}H-^{1}H COSY (600 MHz, DMSO-d$_6$ spectrum of **8**. Blue cross peaks) illustrating $^3J_{\text{H-H}}$ (vicinal) interactions within the tropolone ring. The proton appended to C-1 is a singlet due to the lack of any vicinal interactions. A larger version can be found in Figure S12.

In order to demonstrate the richness of the information provided by FTIR, the students were asked to compare a set of molecules of closely similar structure ranging from phenol, catechol, pyrogallol and purpurogallin, data was available in house (See Supporting Information), which they compared with their own spectra, as advised by Bellamy (1968).[38] Consequently, we used closely matched series of compounds to train students. The carbonyl groups in general were readily identified by the students and are one of the most recognizable and prominent features but not for **8**. Survey results showed that in general the students were confused as to which peaks were important and many, if not warned, tried to assign every peak as is the practice in NMR spectroscopy. Charts are often used by students to interpret spectra that tabulate a functional group adjacent to the highest wavenumber and moving to functional groups assigned downwards to lower numbers. We have found that this has not always proved useful for less experienced students who remain uncertain as to which groups to assign first. Student were asked to cover a print of the spectrum of **8** (and that of an authentic recrystallized **8**) with a blank opaque sheet of paper and moving it upwards to allow them to focus on the most intense bands. Further guidance was given by asking students to assign major peaks using labels A to G, which also facilitated marking. According to Le Fèvre *et al.*[39] **8** shows strong absorption at 1590 cm^{-1} as well as weaker absorption at 1627 cm^{-1}. Students did not manage to deduce that the later was assigned to a carbonyl but were completely and correctly convinced it was there from the DEPT-Q ^{13}C spectrum. Disappointingly, students were unable to identify the 1,2,3-trisubstitution within purpurogallin, using infra-red tables that can be used to detect such substitution patterns[40] since the presence of the carbonyl group overrode such aromatic interactions. The major difficulty found in FT-IR analysis by all students was that some bands overlapped and the carbonyl was weaker in intensity than expected, making it difficult to be certain when making this assignment. However by the end of the course they all became adept in using triangulation between spectroscopy and spectrometry, to affirm or refute a deduction (e.g. NMR shift, IR frequency and a fragment detected in Electron Impact or Ionisation data). A useful alternative was to use an online wizard site designed by Steffen St. Thomas which empirically assigns the five most intense bands as strong (s); medium (m) ; weak (w); variable (v).[41] It uses robust data from important compendia.[42-43] Student FTIR spectra and an interpretive guide can be found in Figures S14-18.

Students were encouraged to run their own mass spectrum independently since training is provided at the onset of the semester; this type of "Buy-in" empowers the student to prepare their own samples and take responsibility for their research. Direct infusion in the electrospray experiment is sufficient to obtain satisfactory spectra (Figure 7) in the negative ionization mode. Training was given in sample preparation which involved three serial dilutions of their product in methanol, all of the students managed to acquire their own spectrum in positive ion mode to obtain a low resolution electrospray experiment using a Waters LCT classic. Only one student (from a group of 20) flooded the instrument, and unable to keep the ion count sufficiently low to prevent saturation and potential damage. Students were trained to always run a nominal or low-resolution experiment before migrating to high-resolution electrospray mass spectrometry experiments. However, the later instrument had an interface that proved challenging (MassHunter) so was only operated by the specialist technical staff.

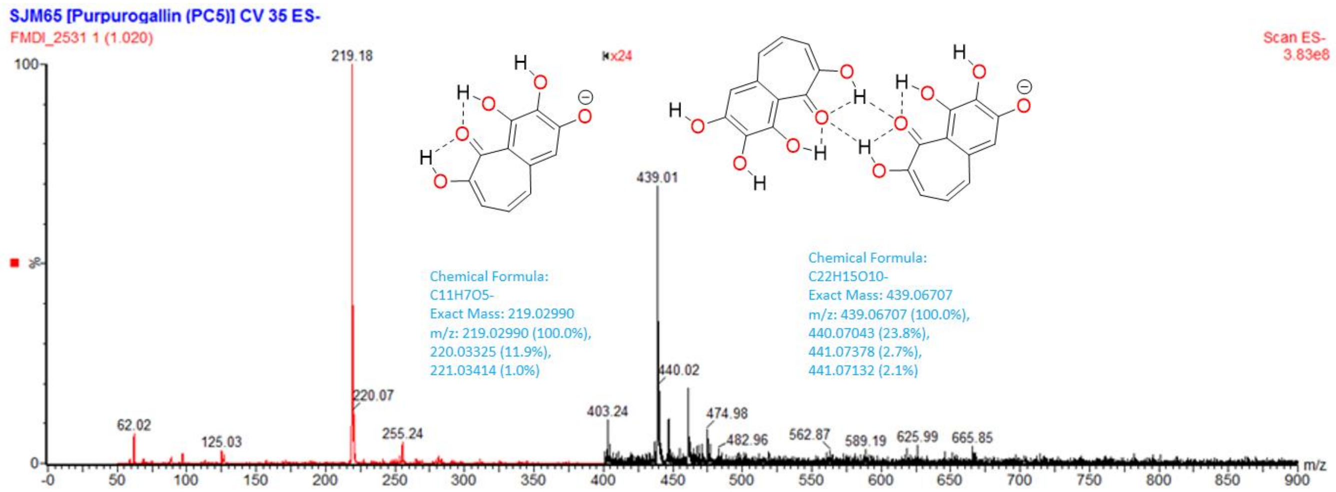


Figure 7. a) The nominal –ve ion electrospray mass spectrum illustrating the presence of the monomer as the dominant species and the anion of the dimeric complex (magnified x24). In all spectra it is assumed that these are anions and not radical anions. A larger version can be found in Supplementary Figure S19.

Figure 7 gives what may at first sight, be inconsistent with **8** and can be used to make the students understand that complexes and adducts are often encountered in contrast to electron ionization experiments. Student results slightly varied here because notably impure or samples stored in soda-glass vials can incorporate sufficient sodium into the sample to give only the sodiated dimeric adduct (See Figure S14). The metal binding ability of **8** is well known[44] and ranges from transition metals to aluminium and has been exploited within medicinal chemistry for targeting a range of metalloenzyme drug targets.[45] Furthermore, this allows students to become familiar with the National Institute of Standards and Technology (NIST) library as well as Spectral Database for Organic Compounds (SDBS) and adds to their structural determination experience.[46] In this case, the students were asked to do a search and found that **8** is not currently in the NIST database but present in SDBS.

All the students ran their own NMR [^{1}H, ^{13}C-DEPT-Q, HMBC, HSQC], FT-IR and MS. This gave these students a sense of ownership and buy-in when compared to a cohort that was simply provided with spectra previously ran. Clearly in terms of education collection of data themselves has proved more beneficial. The practical was also followed by a guided workshop in structural determination and the students enjoyed understanding the application of logic in deducing structures and using a combination of techniques. However, if the molecular formula was not provided, students floundered and about half failed to link the facts together. None failed to solve the structure given the molecular formula or through use of -ve ion mass spectrometry using their own sample. Having been given an accurate molecular formula all students managed to calculate the unsaturation index. This was followed by determination of the presence of a carbonyl peak using ^{13}C NMR and also that there were four OH groups with one strongly hydrogen-bonded using ^{1}H NMR. It was clear to the students the power of 2D NMR to link all the structural pieces of information together. The students always felt engaged when this experiment was presented both in a historical context and that previously the problem had been solved by the application of chemical logic alone and without spectroscopy.

A comparison of structural determination methods

The practical was designed to provide examples of spectroscopic, spectrometric and using single-crystal X-ray data allowing undergraduates to reconstruct and understand bonding arrangements. Integral to the teaching experience was the students understanding of "what constitutes structural determination?" and to compare and contrast various techniques which have an element of assignment approach [NMR, FT-IR, ultra-violet (UV) etc]. This allowed comparison between methods that require combining data to "deduce connectivity" with those that allow direct determination such as X-ray and more recently cryogenic electron microscopy (cryo-EM) which students were asked to read about and has proven useful in structural elucidation of small molecules,[47] as a powerful new tool for confirming difficult structures and revising miss-assignments.[48]

Consequently, students were provided with the published X-ray structures of **8**[49] (provided in Supporting Information) to ascertain using “text wall" software if they could cross relate the data shown with that deduced from their NMR structures. When surveyed during a post-lab class, only a minority of students understood the convention that hydrogen bonding (Figure 8) was depicted by dotted lines (2/20). Comparison was made to another compound containing an intramolecular hydrogen bond, quercetin, to emphasize the point and to indicate the broader scope.

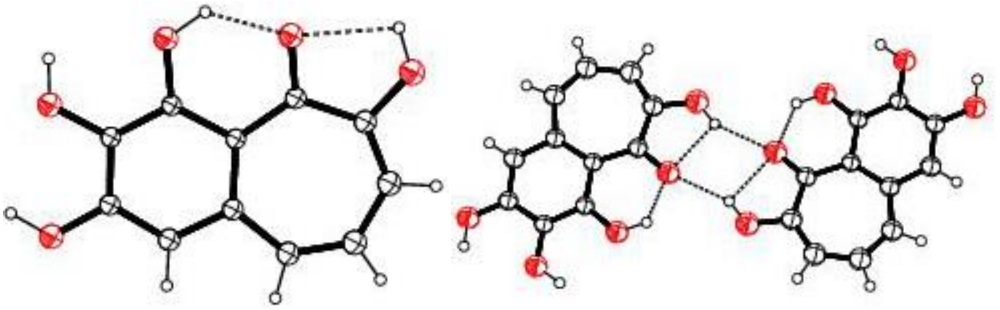


Figure 8. Ortep representations from the crystal structure of **8**.[49] Hydrogen bonding interactions are shown as dotted lines in the monomer and dimer. The C-4 OH and the C=O group is the least exchangeable. See Figure S23 for a computationally derived presentation.

In the same survey, it was determined that all students believed that X-ray structures provided direct evidence of bonding arrangement's but failed to know that the positions of protons are normally assigned, but none provided an answer as to how these positions of protons could be determined; this information had not been given directly in lectures (but in further reading as a pre-practical exercise). When questioned further, finding sufficient study time was a critical factor. Consequently, in recent years, lecture material has been rewritten such that the advantages and limitations of each technique are also critically discussed in a group session using a Socratic method.[50] Also, a reference was made to the seminal work of Dorothy Hodgkin and her work on vitamin B12 and the use of neutrons in determining the position of covalently attached hydrogens and protonation sites as well as more modern investigations comparing X-ray and neutron diffraction studies.[51] One post-course survey in 2018 showed that the women in the cohort appreciated the inclusion of a reference to not only a notable woman in Science who was also a Nobel laureate but one who also encouraged other women into science.[52]

Student Survey

After each group workshop session, a mini student survey was conducted, and any problems were addressed. A final student survey (Example survey questions are in Supporting Information) at the end of the course has consistently generated 80-100% satisfaction (5 year survey, 2014-2019) since the course addresses any shortfalls in knowledge by continuously maintaining a blog inside the course management system. High levels of student satisfaction are found and since all experiments and instrumentation are hands-on whilst supervised by the senior scientific office and the educator, this has prepared many students for both industry and further postgraduate study.

CONCLUSION

The undergraduate practical experiment is a basic organic synthetic practical employing a one-step basic oxidation of a triol to produce a tropone. It is taught using a assisted inquiry approach whereby the students must devise their own laboratory script. In general we found that the overall engagement of the student was high; the students were satisfied that they could create a full protocol from an academic paper. It provides a range of practical and structural elucidation skills allowing transition to more complex problems such as quinine. All students reported that the complexity of the compound was sufficient to allow a deduction in a 2-hour workshop and understood that 2D-NMR spectra were a vital tool in rapid deduction of natural product spectra.

Overall, students enjoyed both elements of the practical, initially learning the history of gall inks and how important they are in history, as well as today, without fully comprehending where the ink came from or how easily it can be produced. Secondly, the analysis segment gave students a first-hand experience using sophisticated analytical instruments such as the NMR, providing them with independence and motivation in their work. Implementing skills, they had previously learnt as well as applying theory of the instruments.

The experiment encouraged students to think how different results can be obtained with small, careful, adjustments to reagents and conditions. Most students struggled at first with the depiction of the NMR spectra, yet this challenged them to conduct further reading outside of lectures and lab classes to complete these tasks, an objective of the instructors.

The experiment gave them valuable laboratory skills even though they used simple synthetic laboratory apparatus, the key to this experiment is its easy adaptability for unexperienced groups and with an interesting color can captivate any audience young or older. Finally, the presence of such compounds in historical documents helped to draw in many students and very few had not heard of Leonardo da Vinci, making this synthesis and spectroscopy conundrum experiment an enjoyable experience.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available on the ACS Publications website at DOI:

Spectra data (PDF)

Notes for Instructors (DOCX)

X-ray data for purpurogallin and quercetin (PDB)

Handouts for Masters, undergraduates and high school (DOCX)

Videos of preparation (MOV)

Assignment flow diagram (EMF)

IR chart (PNG)

Student survey questionnaire (DOCX)

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