Chemistry: Bulgarian Journal of Science Education, Volume 22, Number 1, 2013
Природните науки в образованието

Experiments
Учебен експеримент в природните науки

COLORFUL EXPERIMENTS FOR STUDENTS: SYNTHESIS OF INDIGO AND DERIVATIVES

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Abstract. In order to enhance the interest for organic chemistry among undergraduate students, the synthesis of indigo and three derivatives are described. In this way, students can see the influence of aromatic substituent groups. The syntheses of indigo, 5,5’-indigo disulfonic acid, 5,5’-dibromoindigo, 6,6’-dichloroindigo are proposed.

Keywords: indigo, indigo carmine, 6,6’-dichloroindigo, substituent effect

Introduction

Blue has always been a fascinating color for Humans: it was particularly present in various form of art from the Egyptians using their Egyptian Blue (a calcium copper silicate) for faience (e.g., Bust of Nefertete in 1340 BC) to contemporary Art with the *International Klein Blue* of the painter Yves Klein (Berke, 2007). Mostly this color was found as a mineral form, useless for dyeing fabrics or clothes. An exception is the popular indigo and its brominated derivative the famous Tyrian purple. They are among the oldest organic dyes used in the human history (Balfour-Paul, 1998; Jacquemin et al., 2006; de Melo et al., 2004). The rareness of blue lightfast dyes attracts the attention since centuries or even millennia of almost all cultures around the World (Chinese, Greeks, Indians, Maya, etc.). Surprisingly most of them have developed independently techniques for dyeing fabrics or even made pigments based on the use of plants or animals containing indigo precursors. Effectively, plant indigo or mollusk purple are not present as the pigment in these organisms but as a hidden water soluble and colorless form and the characteristic color appear only after a complex enzymatic and oxidation process. The amazing stability of the indigoids structure explains how these dyes have survived until today in some archaeological remains. We know that indigo from vegetal origin was found in the hands of the ancient Egyptians embalmers or Tyrian purple as a pigment used in wall painting in Greece long before the first millennia BC (Sotiropoulou, 2004).

These properties have retained the attention of many scientists and chemists during centuries trying to understand and resolve the composition of these materials. Finally during the second half of the 19th century the indigoid structure was identified, opening the doors to laboratory synthesis. Later the industrialization of the production of indigo was at the origin of well-known companies such as BASF (Balfour-Paul, 1998).

An important source of indigo came from Asia mostly from the plant *Indigofera tinctoria.* In Europe it came from local species such as woad (*Isatis tinctoria*) during the Middle Age (Jacquemin et al., 2006). With the development of the transoceanic roads, its culture was developed in the West Indies and Americas from the 17th century but became obsolete after the introduction of synthetic indigo at the end of the 19th century (Ferreira et al., 2004; McKee & Zanger, 1991). Today, its production still remains important: about 33’000 tons of indigo are produced each year mostly for the dyeing of clothes (e.g.*,* blue jeans) (Backburn et al., 2009).

The precursors of *Tyrian purple* or *Royal purple* are found in the hypobranchial gland of some Mediterranean shellfishes of the *Muricidae* family. A complex multi-steps process converts them into the colored pigment (Lamsabhi et al., 2005). This extraction was succinctly described by Pliny the Elder in his *Natural History* at the beginning of our era (Haubrichs, 2004; Cooksey, 2001). The small amount of the dye present in the shellfish (< 1 mg) (Ferreira et al., 2004) explains its price (Purple clothes were a reserved privilege of noblemen or Roman emperors (Immings et al., 2001)). In 1909, in a famous publication, the chemist Paul Friedländer mentioned that he had to extract the glands of 12’000 *Bolinus brandaris* (which is equivalent to several hundred pounds) to isolate only 1.4 g of the dye (Friedländer, 1909). This amount was, at this time, just enough for him to determine the exact structure of the purple pigment: 6,6’-dibromoindigo (Immings et al., 2001). It is interesting to note that a dye named *tekhelet* (the “*sacred blue*”) was used by the ancient Jewish community. It was extracted from another mollusc the *Murex trunculus,* a specie that contains brominated and unbrominated precursors contributing to a considerable variability in the resulting color ranging from indigo blue to a deep purple (Hoffman et al., 2010; de Melo et al, 2006).

Origin of the “blue” color

The intriguing characteristic of indigo (Fig. 1, left) is this strong blue color due to its small first excitation energy of about 2 eV in polar solvent (as reference, the colored compounds observed in the range of 400-700 nm have an excitation energy between 1.77 and 3.10 eV) (Jacquemin et al., 2006; Serrano-Andrés & Roos, 1997). Moreover, the position of the lowest absorption band (responsible for the color) varies with the environment (solvatochromism): from 540 nm (red: vapor form) to 588 nm (violet in the apolar solvent CCl_4) or 606 nm (blue in the polar ethanol) (Serrano-Andrés & Roos, 1997).

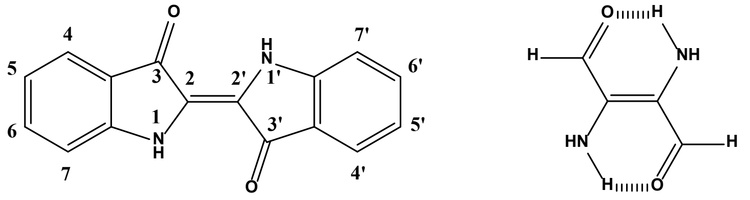


Fig. 1. Sketch of indigo (left) with the numbering position and its H-like chromophore (right) (Jacquemin et al., 2006)

The origin of the color seems to be due to the conjugated system of the indigo without the benzene ring (Fig. 1, *right*) (Cooksey, 2001). An explanation of this blue color relies on the strong internal hydrogen bonds (Fig. 1, *right*) (de Melo et al., 2004) between both oxygen of the carbonyls and nitrogen that favor a C^{+}-O^{-} structure (excited state) and lower the highest HOMO-LUMO gap of the molecule (Jacquemin et al., 2006). Those hydrogen bonds are especially strong since they form 6-membered rings and allow a good overlapping of the orbitals (Nagasawa et al., 2004).

X-ray data of indigo showed also intermolecular hydrogen bonds, each molecule of indigo being attached to four others (Christie, 2001). This conformation with strong bonds explains the bathochromism of the molecule, its low solubility and its high melting point (390-392 °C) (Christie, 2001).

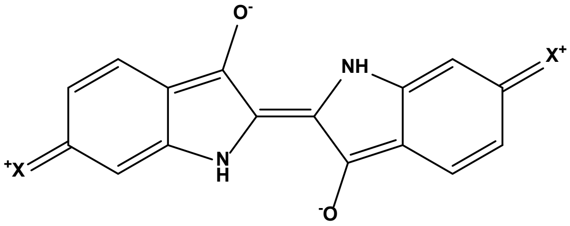
Influence of the aromatic substituent

The role of the aromatic substituent groups on the modification of the absorption of the indigoids dyes has been already described by Sadler (1956). It was explained that substituent groups may affect the energy level of the excited state of the indigo either by increasing or diminishing electron release from the nitrogen group or electron withdrawal by the carbonyl group (Sadler, 1956; Christie, 2001).

It explains why 6,6'-dibromo- or 6,6'-dichloroindigo are red solids: the contribution of the halogen will decrease the flow of electrons from the nitrogen to the carbonyl group creating a hypsochromic shift (Fig. 2).

On the contrary, the 5,5'-dibromoindigo will increase the availability of electrons to the imine and by electronic cascade to the C=O group. The energy difference will be smaller between the excited and the ground state (bathochromic shift).

Multiple substitutions of halogens on indigo may have different effects on the color: 6,6',7,7'-tetrabromoindigo shows the same absorption maxima as 6,6'-dibromoindigo (590 nm) while 5,5',6,6'-tetrabromoindigo has an absorption maxima at 605 nm (Sadler & Warren, 1956).



X = F, Cl, Br, I, -OMe

Fig. 2. Effect of electron donating groups on indigo (Sadler, 1956)

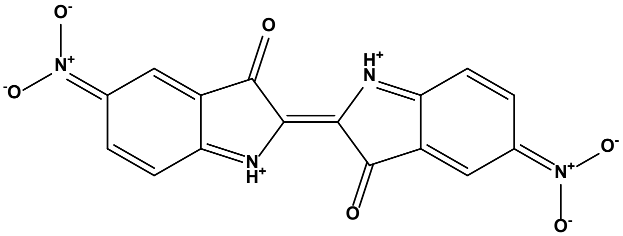


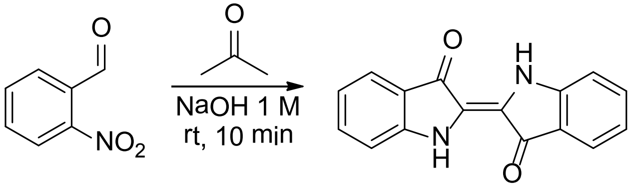
Fig. 3. Effect of electron withdrawing groups on indigo (Sadler, 1956)

Due to the electron-withdrawing nature of their substituent groups, 5,5'-dinitroindigo and 6,6'-dinitroindigo have the exact opposite behavior as their bromo analogs (Fig. 3). Their different absorption spectra data can be read in Table 1.

Table 1. Absorption spectra data of indigo with its bromo- and nitro-derivatives (Sadler, 1956)

	Wavelength maxima of the absorption spectra of indigoids in 1,1,2,2 tetrachloroethane at 20 °C [nm]	
	5,5'	**6,6'**
H-Indigo	605	605
Dinitroindigo	580	635
Dibromoindigo	620	590

Indigo carmine confirms also the importance of the position: located in 5,5'-position, the sodium salt of the disulfonic acid does not affect the color. Indigo carmine is in fact blue (λ_{abs} = 610 nm in H_2O (Nagasawa et al., 2004)) like its parent compound indigo.



Scheme 1. Baeyer-Drewson synthesis of indigo

An interesting publication about indigoids synthesis was already reported by Schatz (2001). In his paper, students could see the relation between the bromine positions (molecular level) on the color of the indigoid dye (macroscopic level). In our paper, the syntheses of indigo and three derivatives (5,5'-dibromoindigo and 5,5'-disulfonic acid indigo (both blue) and 6,6'-dichloroindigo (purple)) were proposed.

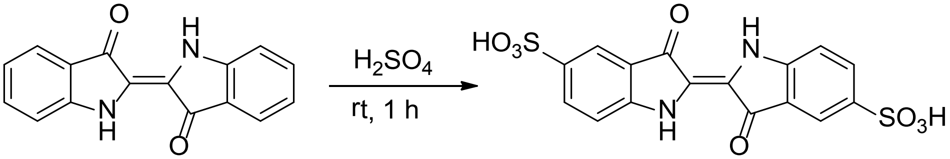
Experiments and discussion

Indigo was prepared following the modified procedure published by McKee & Zanger (1991) of the classical Baeyer-Drewson synthesis using acetone and sodium hydroxide (Baeyer & Drewson, 1882) -Scheme 1.

The product obtained was blue with purple sheens (Fig. 4).

Even if ^{1}H-NMR spectra can be recorded (Guengerich et al., 2004; Cooksey, 2001), it is more convenient to record the IR spectra due to the low solubility of indigo. Char-

Fig. 4. Indigo synthesized by an undergraduate student



Scheme 2. Synthesis of indigo carmine from indigo

acteristic peaks should be observed for the N-H stretch (3250-3400 cm^{-1}) and the C=O stretch (1620-1640 cm^{-1}) (Tanoue et al., 2004).

Indigo carmine can be obtained from this synthesized indigo and sulfuric acid (Shadi et al., 2004) - Scheme 2.

Used as food colorant, the sodium salt of the 5,5'-indigodisulfonic acid is also used as a pH indicator with two transitions at pH = 11.4 (green) and pH = 13 (yellow). Fig. 5 illustrates the progressive deprotonation of indigo carmine with sodium hydroxide. More recently, indigo carmine received some interest as a material for rechargeable batteries (Yao et al., 2010).

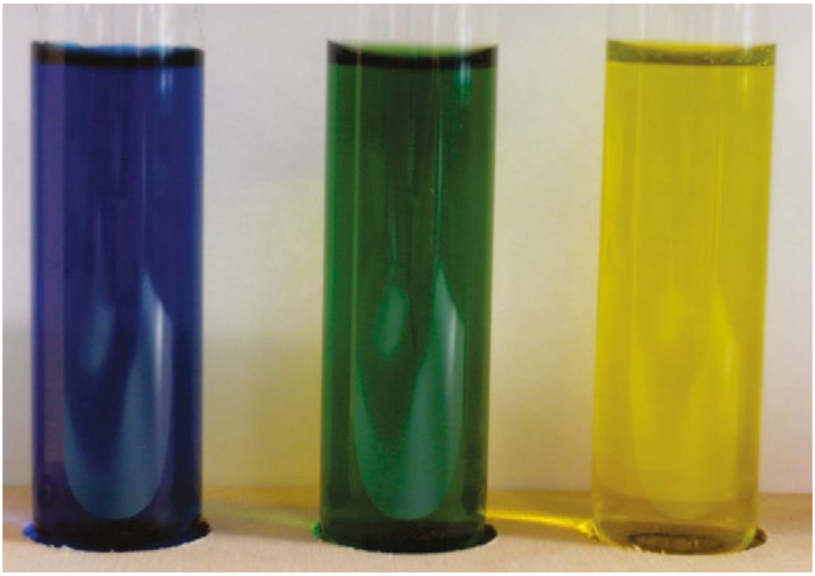
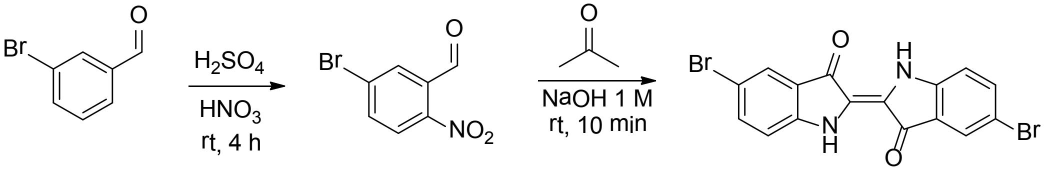


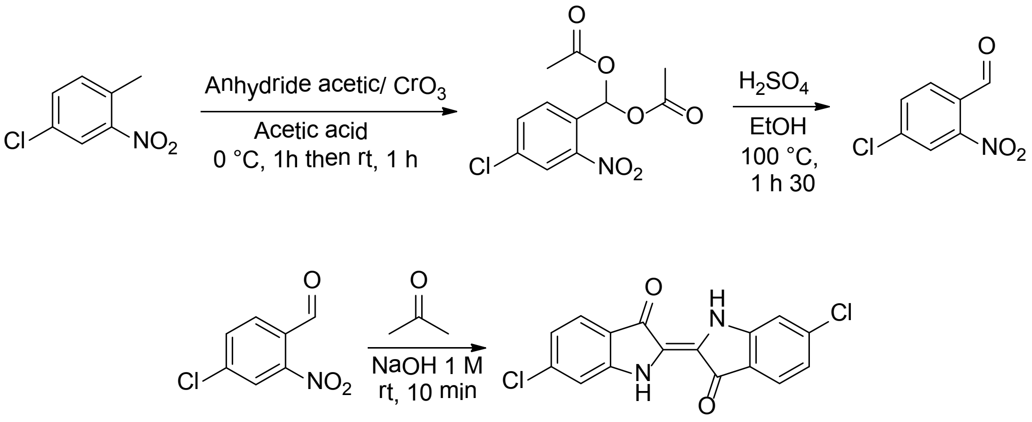
Fig. 5. (from left) Colors of an aqueous solution of indigo carmine at pH = 1, pH= 11-13 and pH = 14



Scheme 3. Synthesis of 5,5’-dibromoindigo

Described as “greener” than indigo by Schatz (2001), 5,5’-dibromoindigo is “blue-shifted” compared to indigo (Sadler & Warren, 1956). One of the first syntheses was achieved by Friedländer et al. (1912) starting from 3-bromobenzoic acid. The 5,5’-dihalogenoindigo were also prepared from the corresponding halogenoindoles (Tanoue et al., 2004). In our experiments, the nitration of 3-bromobenzaldehyde with nitric acid in sulfuric acid provided easily the desired 5-bromo-2-nitrobenzaldehyde (Hu et al., 2003). The condensation of this aldehyde using the Baeyer-Dawson reaction gave us the expected blue 5,5’-dibromoindigo (Scheme 3).

The most famous 6,6’dihalogenoindigoid is the 6,6’-dibromoindigo, the Tyrian purple. Many syntheses of this compound have been published starting with different raw materials (Wolk & Frimer, 2010). In most of them, the 4-bromo-2-nitrobenzaldehyde was the key molecule, giving access to the Tyrian purple by the classical Baeyer-Drewson condensation. Pinkney & Chalmers (1979) proposed a long but interesting synthesis starting from 4-methylphenylamine obtaining 4-bromo-2-nitrotoluene in two steps followed by an oxidation with CrO_3 to give the desired aldehyde.



Scheme 4. Synthesis of 6,6’-dichloroindigo

As the color of 6,6'-dichloroindigo is very similar to Tyrian purple and in order to avoid the fastidious multistep synthesis of the brominated raw material, we suggested the synthesis of the chlorinated derivative starting from the cheap and commercial 4-chloro-2-nitrotoluene.

Maybe due to problems during the filtration, the yield was low for the formation of the diacetal (19%). The second step gave us the pure aldehyde after a silica gel column chromatography. 4-chloro-2-nitrotoluene was obtained with a better yield compared to the 4-bromo-2-nitrotoluene prepared by Pinkney & Chalmers (1979) (56% against 24% for Pinkney & Chalmers) (Scheme 4). The analyses of the aldehyde were in accordance with the literature (Scott et al., 2006).

An other approach to synthesize of 4-bromo-2-nitrobenzaldehyde was envisaged: starting from 4-bromo-2-nitrotoluene where the methyl group would be oxidized via the formation of an enamine with pyrrolidine in *N,N*-dimethylformamide-dimethylacetal. The formed enamine would be cleaved by oxidation with $NaIO_4$ (Scott et al., 2006; Seo et al., 2006). Good yields (77% - 93%) were obtained in literature but the price of 4-bromo-2-nitrotoluene makes the synthesis too expensive for students. The same procedure was employed in several papers for 4-chloro-2-nitrotoluene with success (Scott et al., 2006; Caron et al., 2003) but it was thought that the use of CrO_3 would be more affordable for the majority of laboratory.

Experimental

Hazards

Students have to wear protective glasses, lab coats and gloves. Diethyl ether is a highly flammable and volatile liquid that may form peroxides upon storage. It is also harmful by inhalation, ingestion, or skin contact, causing irritation, dizziness, drowsiness or unconsciousness with prolonged exposure. Ethanol is a flammable, volatile liquid. Ethyl acetate and pentane are volatile, flammable liquids that are also irritants. NaOH 1 M, HNO_3 65%, H_2SO_4 96%, acetic acid, acetic anhydride are corrosive and may cause severe burns if in contact with skin or eyes. Acetic anhydride and acetic acid are flammable. 2-nitrobenzaldehyde (R36/37/38, S24/25) is skin and eyes irritant and hazardous in case of ingestion or inhalation, 3-bromobenzaldehyde (R22) is harmful if swallowed and 4-chloro-2-nitrotoluene (R20/21/22, R36/37/38, S26, S36/37/39) is harmful in case of inhalation, of contact with skin or swallowed and irritating to eyes and skin. Chromium oxide (VI) (R35, R35, R43, R49, R50/53, R8, S45, S53, S60, S61) is highly toxic and may cause cancer by inhalation (O'Shea et al., 2012).

Materials and methods

Starting compounds and solvents were purchased from Sigma-Aldrich/Fluka or Acros and were used without further purification. TLC silica plates were purchased from

Merck. TLC plates were developed with $KMnO_4$. Column chromatographic separations were carried out using 230-400 mesh silica gel. ^1H spectra were recorded (as indicated) on either a Bruker 300 MHz or 400 MHz spectrometer and are reported as chemical shifts (δ) in ppm relative to TMS ($\delta = 0$). Spin multiplicities are reported as a singlet (s), doublet (d) or triplet (t) with coupling constants (J) given in Hz, or multiplet (m). Broad peaks are marked as br. All NMR spectra were recorded in $CDCl_3$. IR spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer (ATR, Golden Gate). Melting points were recorded in open capillary tubes with a Buchi B-550 melting points apparatus and were uncorrected.

Synthesis of indigo

This procedure was adapted from McKee & Zanger (1991) where they used the Baeyer-Drewson reaction.

2-nitrobenzaldehyde (1.0 g) was dissolved in acetone (20 mL) and H_2O (35 mL) was added. NaOH 1M (5 mL) was added dropwise and the solution was stirred 5-10 min. The precipitate was filtered and washed with H_2O until the filtrate was colorless. The solid was then washed with ethanol (2 x 20 mL).

The precipitate was collected and dried at 40 °C under reduced pressure (10-20 mbar) to remove the solvents. A blue product (640 mg, 2.44 mmol, 37%).was collected.

IR (cm^{-1}) 3259, 1710.6, 1610, 1583, 1481, 1460, 1390, 1314, 1297, 1193, 1171, 1123, 1064, 877, 858, 790, 747, 697, 636.

Synthesis of 5,5-disulfonic acid indigo (indigo carmine)

The procedure was adapted from Shadi et al. (2004).

The indigo previously synthesized (23 mg) was dissolved in H_2SO_4 96% (16 mL). The reaction mixture was stirred for 1 h at 20 °C. The mixture was cooled at 0 °C and NaOH 2 M was added slowly until pH reached 7. Then NaOH pellets were added to the solution to reach pH = 13-14.

Syntheses of 5,5-dibromindigo and 2-nitro-5-bromobenzaldehyde

The procedure to synthesize 2-nitro-5-bromobenzaldehyde was taken from Hu et al. (2003).

3-bromobenzaldehyde (9.25 g, 5.00 mmol) was added portion-wise during 10 min to a stirred mixture of HNO_3 65% (5.0 mL) and H_2SO_4 96% (60 mL) cooled in an ice bath. After 4 h at room temperature, the solution was poured into ice (200 mL) the precipitate was filtered and washed with H_2O (100 mL). The crude material (about 10 g) could be used directed for the next step without purification.

However, students can purify a certain amount by silica gel chromatographic column. A sample of 3.2 g of crude material was dissolved in CH_2Cl_2 (50 mL) and 40 mL of silica were added. CH_2Cl_2 was removed under reduced pressure. A chromatographic column was prepared using 150 mL of silica (about 60 g) and eluted with Pentane/EtOAc, 95:5. The evaporated mixture of the crude material and silica was deposed on the top of the column and the elution started. Fractions of 50 mL were collected. The product was collected from fractions 12 to 17. Pure 5-bromo-2-nitrobenzaldehyde (2.2 g) was obtained.

Mp = 70-72 °C; **Rf** =0.45 (Pentane/EtOAc, 95:5); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 10.41 (s, 1H), 8.09 – 7.98 (m, 2H), 7.88 (dd, J = 8.6, 2.2 Hz, 1H); **IR (cm^{-1})** 3094, 1689, 1599, 1560, 1506, 1399, 1380, 1345, 1302, 1258, 1176, 1092, 1067, 898, 880, 840, 754, 713, 672, 647.

Synthesis of 5,5'-dibromoindigo

Crude 2-nitro-5-bromobenzaldehyde (2.25 g, 9.8 mmol) was dissolved in acetone (50 mL) and H_2O (70 mL) was added. NaOH 1 M (12 mL) was added dropwise and the solution was stirred 10 min. The precipitate was filtered and washed with H_2O until the filtrate was colorless. The solid was then washed with ethanol (2 x 40 mL) and acetone (40 mL) (to remove any trace of 3-bromobenzaldehyde).

The precipitate was collected and dried at 40 °C under reduced pressure (10-20 mbar) to remove the solvents. A blue product (1.02 g, 2.40 mmol, **24%**) was collected.

IR (cm^{-1}) 3280, 1712, 1626, 1603, 1440, 1385, 1301, 1255, 1217, 1179, 1137, 1112, 1077, 1040, 884, 819, 780, 713, 700, 643.

Syntheses of 6,6'-dichloroindigo and 4-chloro-2-nitrobenzylidene diethanoate

The procedures were adapted from Pinkney & Chalmers (1979).

In a three-necked flask equipped with a mechanical stirrer and a thermometer, 4-chloro-2-nitrotoluene (16 g, 93 mmol) was dissolved in pure glacial acetic acid (40 mL) and acetic anhydride (220 mL) was added to the solution followed by sulfuric acid (21 mL). The solution was cooled at 0-5 °C and a solution of chromium oxide CrO_3 (25 g) dissolved in H_2O(25 mL) and glacial acetic acid (100 mL) was added over 1 h maintaining the temperature between 5 and 10 °C. The mixture was stirred for a further hour and poured into iced water (1.5 L). The yellowish solid which separated out was filtered off, washed with H_2O until the filtrate was colorless and dried at low pressure (vacuum pump). A pure white powder was collected (5.3 g, 18 mmol, **19%**).

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.14 (s, 1H), 8.04 (s, 1H), 7.67 (s, 2H), 2.14 (s, 6H); **IR (cm^{-1})** 1754, 1613, 1526, 1480, 1431, 1372, 1346, 1195, 1120, 1059, 999, 953, 909, 885, 827, 764, 740, 678, 655, 607.

Synthesis of 4-chloro-2-nitrobenzaldehyde

4-chloro-2-nitrobenzylidene diethanoate (5.2 g, 18 mmol) was boiled with ethanol (40 mL) and sulfuric acid (2.7 mL) for 90 min. After cooling at 20 °C, the mixture was treated with H_2O (140 mL) and the aqueous phase was extracted with diethyl ether (2 x 100 mL). The organic phases were washed with saturated $NaHCO_3$ (100 mL) and dried over $MgSO_4$. After evaporation of the solvents, 3.2 g of crude material were obtained.

The crude material was dissolved in CH_2Cl_2 (about 50 mL) and 60 mL of silica were added and the solvent evaporated. A chromatographic column was prepared with 350 mL of silica and eluted with Pentane/EtOAc, 95:5. The crude material was deposed on the top of the column and elution started. Every fraction of 50 mL was collected. Product came out from fraction 15 to 25. The 4-chloro-2-nitrobenzaldehyde was obtained as a yellowish solid (1.9 g, 10 mmol, **56%**).

Mp = 64-66 °C; **Rf** = 0.33 (Pentane/EtOAc 95:5); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 10.38 (s, 1H), 8.10 (d, J = 1.8 Hz, 1H), 7.94 (d, J = 8.3 Hz, 1H), 7.76 (d, J = 7.0 Hz, 1H); **IR (cm^{-1})** 3089, 2920, 1808, 1692, 1595, 1520, 1404, 1387, 1344, 1254, 1188, 1151, 1110, 1067, 905, 887, 841, 817, 754, 734, 677, 628.

Synthesis of 6,6'-dichloroindigo

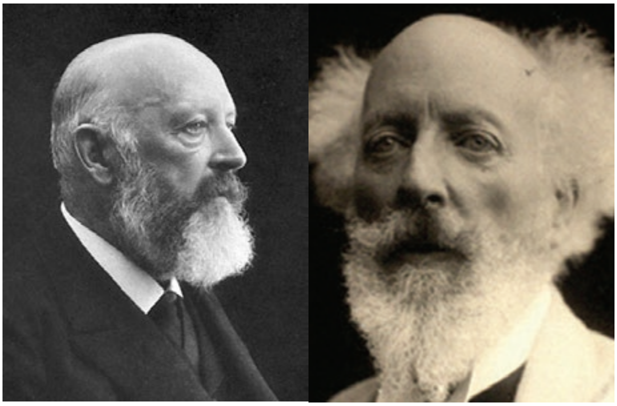
4-chloro-2-nitrobenzaldehyde (500 mg, 2.70 mmol) was dissolved in acetone (11 mL) and H_2O (14 mL) were added. NaOH 1 M (3 mL) was added and the solution was stirred for 10 min. The precipitate was filtered and washed with H_2O until the filtrate was colorless. The solid was then washed with ethanol (2 x 20 mL).

The precipitate was collected and dried under reduced pressure (10-20 mbar) to remove the solvents. A purple product (200 mg, 0.604 mmol, **22%**) was collected.

IR (cm^{-1}) 3259, 1710.6, 1610, 1583, 1481, 1460, 1390, 1314, 1297, 1193, 1171, 1123, 1064, 877, 858, 790, 747, 697, 636.

Conclusion

This project was developed to allow second-year students in bachelor of chemistry to work independently in an organic laboratory. They can familiarize themselves with new analytical methods (^{1}H NMR and IR spectra) and synthesize colorful molecules. It requires no particular chemical skills or harsh conditions. All reactions were conducted



Adolf von Baeyer (1835-1917) and Vigo Drewsen (1830-1888)

without inert gas. Purification of materials is relatively easy and all aromatics compounds can be detected with a UV lamp. This project involves simple reactions (electrophilic addition) and more complicated mechanisms (Baeyer-Drewson reaction).

Acknowledgments. The authors thank S. Torche and A. Zumbuehl for technical support, M. Holme for English assistance and the University of Geneva for funding.

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