# Reaction of Naphthalene-2,3-dicarbaldehyde with Cyanide; A Unique Oxidative Condensation Product



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Naphthalene-2,3-dicarbaldehyde (NDA), a reagent used for the fluorescent detection of amino acids in the presence of cyanide, self-condenses in the presence of cyanide ion and methanol at room temperature to yield a unique crystalline product 2, 15-hydroxybenzo[*g*]benzo[6,7]isochromeno[4,3-*c*]isochromen-7(15*H*)-one. The product is proposed to result from facile air oxidation of NDA to a methyl ester in combination with benzoin condensation. Product 2 does not to form in the absence of air. The gHMBC spectrum of 2 distinguishes it from a possible alternative isomeric condensation product.

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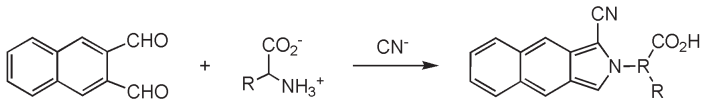
## Introduction.

Naphthalene-2,3-dicarbaldehyde (NDA) **1** in the presence of cyanide was first introduced as a reagent for the fluorescence detection of amino acids and peptides by Carlson *et al*. in 1986 [1,2]. The reagent has been shown to react rapidly with amino groups to form stable, highly fluorescent 2-substituted 1-cyanobenz[*f*]isoindoles as shown below. Since that time, NDA has become a widely used analytical reagent for amino acid and peptide analysis by HPLC [3] and capillary electrophoresis [4].



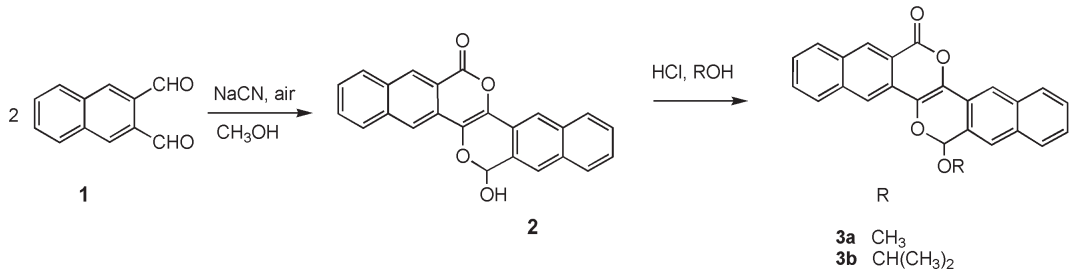

NDA isoindole

As initially reported, sodium cyanide is added to a methanolic solution of NDA, immediately followed by an aqueous solution of the amino acid [1]. The formation of a pale yellow solution after addition of cyanide but *prior* to addition of amino acid was reported, but no characterization of possible side products was made. We report here the structural characterization of the condensation product, **2**, 15-Hydroxybenzo[*g*]benzo-[6,7]isochromeno-[4,3-*c*]-isochromen-7(15*H*)-one, that forms when NDA is reacted with cyanide *in the absence of amino acid*, as shown in Scheme 1. The product is isolated within 10 minutes reaction time at room temperature as a yellow crystalline product. It conveniently reacted to the full acetals **3a** and **3b** in HCl and alcohol.

## Structure Proof of **2**.

The structure of **2** is supported by its IR spectrum, elemental analysis, mass spectrum, and NMR spectra including $^1$H, $^{13}$C, gCOSY (Figure 1), NOESY, gHSQC (Figure 2), and gHMBC (8 Hz and 3 Hz, Figure 3-5)

Scheme 1




**1** **2**

Condensation of NDA in the presence of air and cyanide in methanol.

spectra. Assignments of all $^{1}H$ resonances and all but two $^{13}C$ resonances are made and are discussed below.

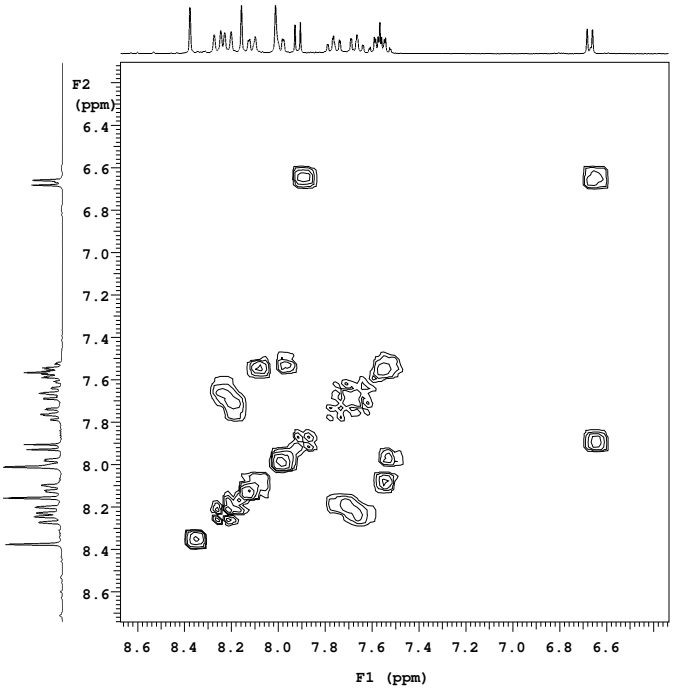



Figure 1. gCOSY Spectrum of **2** in DMSO-$d_6$

relates with 7.65 in gCOSY. Therefore H-10 is 7.65 and C-10 is 127.5. H-10 is coupled to 7.75 (gCOSY) so H-11 is 7.75 and C-11 is 130.1. H-11 is correlated to 8.20. Therefore, H-12 is 8.20 and C-12 is 128.3. Irradiation of 8.36 singlet results in positive NOE at 8.20 so H-13 is 8.36 and C-13 is 119.9

NMR Assignments of C-H Groups on Right Side of Molecule.

Irradiation of H-15 at 6.66 shows a positive NOE at 8.00. Therefore H-16 is 8.00 and C-16 is 125.3. H-5 must be 8.15 and C-5 is 118.1. In the gHSQC, H-2 and H-3 are at 7.55, which correlates to 126.7, and 7.58, which correlates to 127.2.

Assignments of Quaternary Carbons of Left Side of Molecule using gHMBC.

In Figure 3, H-8 shows 3-bond correlations with C-7 (C=O) carbon at 160.1, with C-9 at 129.8, with 12a and 13a at 127.9 and 135.8. H-9 also shows a 3-bond correlation to 135.8. Therefore C-12a is 135.8 and C-13a is 127.9. C-9 also correlates to 132.5 (C-8) and 130.1, which must be C-11. Besides C-12 at 128.3, H-13 correlates to 119.0 and a broader peak at 131.5. The center of the peak at 131.5 has no corresponding carbon, as shown in Figure 4, and is probably an overlap of 130.9 and 132.0. 119.0, 130.9, 132.0 correspond to C-7a, C-8a, C-13b. The HMBC spectrum (3

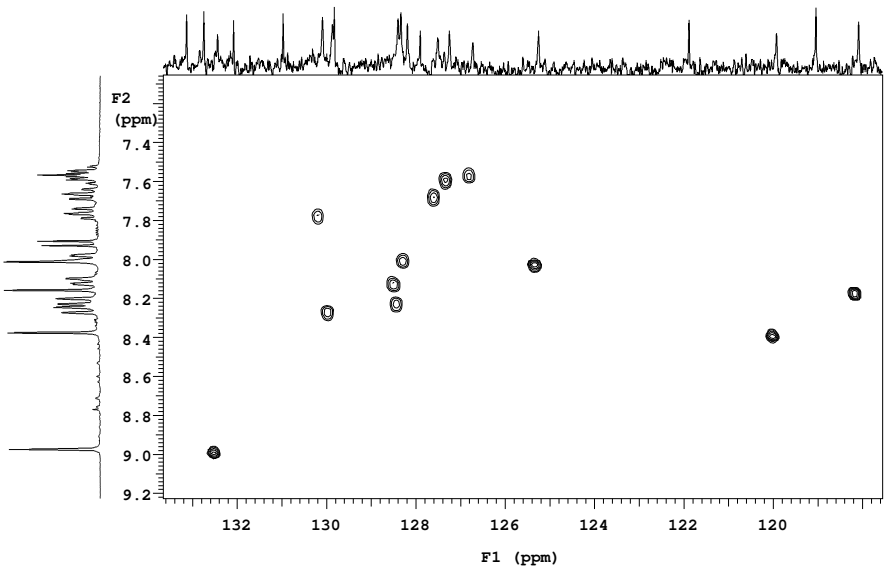



Figure 2. gHSQC Spectrum of **2** in DMSO-$d_6$

NMR Assignments of C-H Groups on Left Side of Molecule.

Irradiation of H-8 at 8.96 shows a positive NOE at 8.25. Therefore H-9 is 8.25 and C-9 is 128.8 (gHSQC). H-9 correlates with 7.65 in gCOSY. Therefore H-10 is 7.65 and C-10 is 127.5. H-10 is coupled to 7.75 (gCOSY) so H-11 is 7.75 and C-11 is 130.1. H-11 is correlated to 8.20. Therefore, H-12 is 8.20 and C-12 is 128.3. Irradiation of 8.36 singlet results in positive NOE at 8.20 so H-13 is 8.36 and C-13 is 119.9

NMR Assignments of C-H Groups on Right Side of Molecule.

Irradiation of H-15 at 6.66 shows a positive NOE at 8.00. Therefore H-16 is 8.00 and C-16 is 125.3. H-5 must be 8.15 and C-5 is 118.1. In the gHSQC, H-2 and H-3 are at 7.55, which correlates to 126.7, and 7.58, which correlates to 127.2.

Assignments of Quaternary Carbons of Left Side of Molecule using gHMBC.

In Figure 3, H-8 shows 3-bond correlations with C-7 (C=O) carbon at 160.1, with C-9 at 129.8, with 12a and 13a at 127.9 and 135.8. H-9 also shows a 3-bond correlation to 135.8. Therefore C-12a is 135.8 and C-13a is 127.9. C-9 also correlates to 132.5 (C-8) and 130.1, which must be C-11. Besides C-12 at 128.3, H-13 correlates to 119.0 and a broader peak at 131.5. The center of the peak at 131.5 has no corresponding carbon, as shown in Figure 4, and is probably an overlap of 130.9 and 132.0. 119.0, 130.9, 132.0 correspond to C-7a, C-8a, C-13b. The HMBC spectrum (3 Hz, Figure 5) shows a clear correlation to 130.9 (apparent de-emphasis of 132.0). H-12 also shows a correlation to 132.0 (shoulder) and therefore C-8a must be 132.0. H-12 correlates to 127.5, which must be C-10. The only carbons

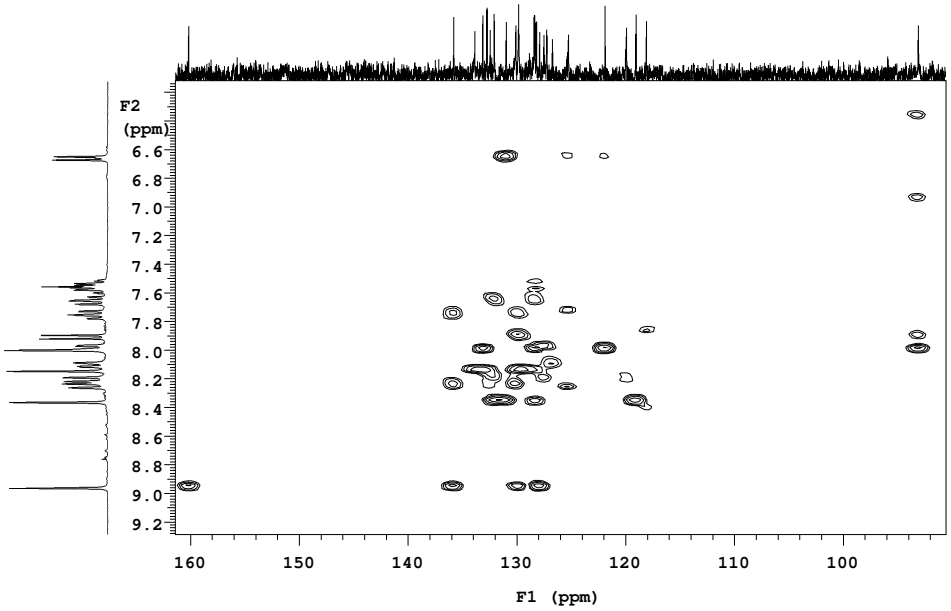



Figure 3. gHMBC Spectrum of **2** in DMSO-$d_6$

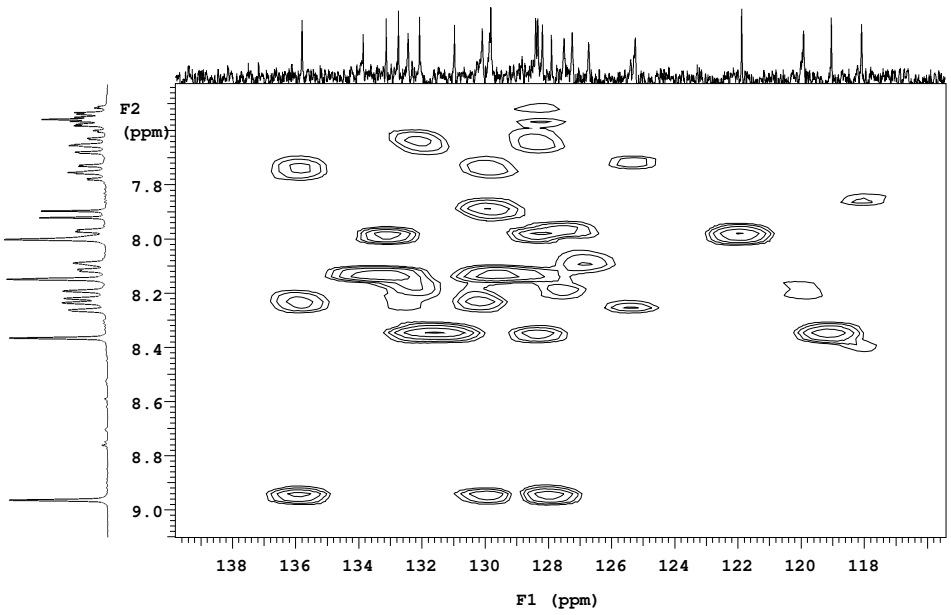



Figure 4. Expanded gHMBC Spectrum (8 Hz) of **2** in DMSO-$d_6$

left unassigned on the left side are C-7a and C-13b, which must be 119.0 and 130.9. The more downfield carbon at 130.9 is logically assigned to C-13b due to the oxygen directly bonded to it. Additionally, the anomeric H-15 correlates to 130.9, confirming it as C-13b.

Assignments of Quaternary Carbons of right side of Molecule using gHMBC.

H-15 correlates with 130.9 (C-13b), 125.2 (C-16), and 121.9, which must be C-5a. H-16 correlates with 93.1 (C-15), 128.2 (C-1), 121.9 (C-5a) and 133.1, which must be C-4a. H-5 correlates to what appears to a broad peak at 133.2, which are probably two overlapping peaks. gHMBC (3 Hz, Figure 3b) clearly shows the correlations of H-5 with 129.8 (apparent de-emphasis of adjacent peak). H-OH shows a two-bond correlation to C-15 (93.1) but also a 3-bond corre-

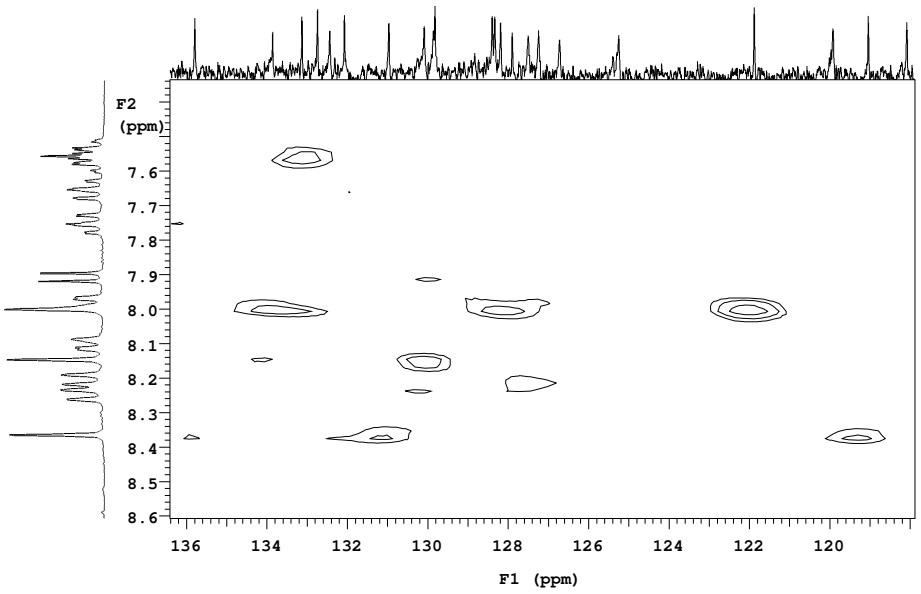
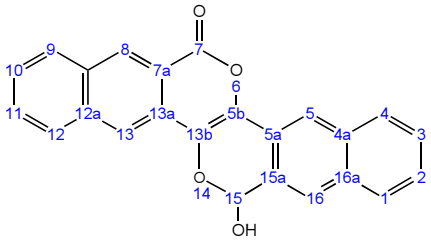



Figure 5. Expanded gHMBC Spectrum (3 Hz) of **2** in DMSO-$d_6$

lation to 129.8. Therefore C-15a must be 129.8. H-4 shows only a single correlation to 126.7, which is a CH carbon that must be C-2, assuming a 3-bond correlation. This leaves 127.2 as C-3. Finally, 132.7 and 133.8 remain unassigned and must be C-5b and C-16a. Assignments are summarized in Table 1 and Figure 4.

Table 1

NMR Correlations for **2** using $^{1}H$, $^{13}C$, NOE, gCOSY, gHSQC, gHMBC




| $\delta_H$, ppm | H-# | $J_{HH}$ | gCOSY | C atoms separated by One Bond $\delta_c$ | C atoms separated by Three Bonds $\delta_c$[a] |
|---|---|---|---|---|---|
| 8.96(s) | H-8 | -- | | 132.4 | 127.9, 129.8, 135.8, 160.1 |
| 8.36(s) | H-13 | -- | | 119.9 | 119.0, 128.3, (130.9,132.0) |
| 8.25(d) | H-9 | 8.3. 1.3 | 7.65 | 129.8 | 130.1, 132.4 |
| 8.20(d) | H-12 | 8.3, 1.3 | 7.75 | 128.3 | 119.9, 127.5, 132.0(shoulder) |
| 8.15(s) | H-5 | -- | | 118.1 | 129.8 (gHMBC 3 Hz, Fig 3b) |
| 8.10(dd) | H-4 | 8.3, 1.3 | 7.58 | 128.4 | 126.7 |
| 8.00(s) | H-16 | -- | | 125.3 | 93.1, 121.9, 128.2, 133.1 |
| 7.98(dd) | H-1 | 8.3, 1.3 | 7.55 | 128.2 | 127.3 (shoulder) |
| 7.91(d) | H-OH | 6.9 | 6.66 | 129.8 | |
| 7.75(ddt) | H-11 | 1.3, 7.0, 8.3 | 7.65, 8.20 | 130.1 | 135.8, 129.8 |
| 7.65(dt) | H-10 | 1.3, 7.0, 8.3 | 7.75, 8.25 | 127.5 | 128.3, 132.0 |
| 7.58(dt) | H-3 | 1.3, 7.0, 8.3 | 7.55, 8.10 | 127.2 | 133.1 (gHMBC 3 Hz, Fig 3b) |
| 7.55(dt) | H-2 | 1.3, 7.0, 8.3 | 7.58, 7.98 | 126.7 | -- |
| 6.66(d) | H-15 | 6.9 | 7.91 | 93.1 | 121.9, 125.2, 130.9 |

[a] All correlations are from gHMBC (8 Hz, Figure 3a) unless noted.

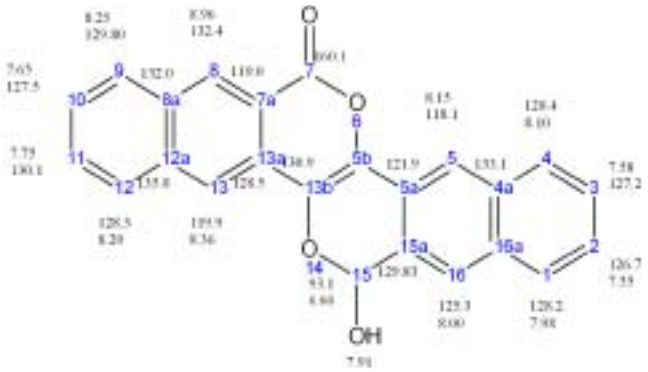
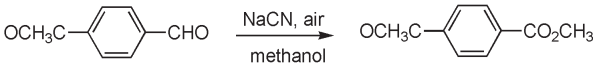


Figure 6. Summary of NMR assignments of **2**. Carbons 5b and 16a are 132.7 and 133.8 ppm.

Reaction Pathway.

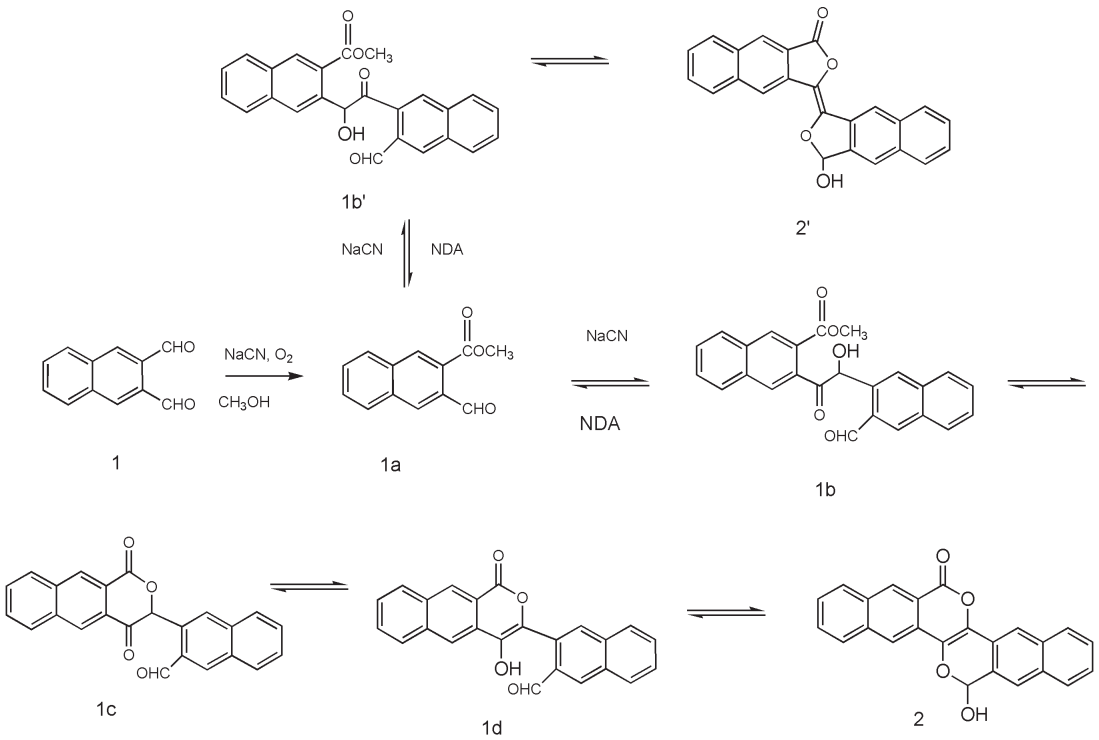
The stoichiometry of the reaction demands that oxidation of NDA takes place. The reaction conducted with exclusion of air did not yield **2**, but unidentified products, demonstrating that formation of **2** requires the presence of air. Thus we can propose a reaction pathway, shown in Scheme 2, which involves (1) air oxidation of NDA to the methyl ester **1a**, catalyzed by cyanide, (2) benzoin condensation to **1b**, (3) cyclization to lactone **1c**, (4) enolization to **1d**, and finally (5) hemiacetal formation to **2**. Steps 2-5 are reversible.

We have no direct spectroscopic evidence for intermediate **1a**; however, it is established that aromatic aldehydes oxidize to form methyl esters in methanol in the presence of manganese dioxide and cyanide [5,6]. This oxidation was shown to proceed by oxidation of a cyanohydrin to an acyl cyanide, followed by methyl ester formation. One can envision a similar reaction here, except with air as oxidizing agent, although it is surprising that oxidation takes place so readily under such mild conditions. Under identical reaction conditions, we found that 2-naphthaldehyde yields less than 5% methyl ester (NMR yield) in 20 minutes, suggesting that only activated aldehydes, such as NDA, are efficiently air oxidized to methyl esters in this time frame. In support of this idea, we found that 4-acetylbenzaldehyde was quantitatively reacted to methyl 4-acetylbenzoate in less than 20 minutes at room temperature.




All of the steps in the proposed reaction Scheme 1 are reversible except methyl ester formation. Thus **2'** could form from the alternative benzoin intermediate **1b'** with a similar reaction pathway as proposed for structure **2**.

Scheme 2

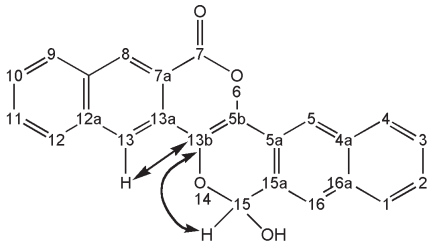



Proposed Reaction Scheme for Formation of **2**

Products **2** and **2'** would be difficult to distinguish by $^{13}C$ and $^{1}H$ spectra alone according to ACD NMR predictive software [7].

Proof of Structure **2** Versus **2'**.

They can be distinguished by considering that H-13 at 8.36 ppm shows a 3-bond correlation with a C-13b at 130.9 (gHMBC, Figure 3). In addition, H-15 at 6.66 ppm also correlates with C-13b. This is possible for structure **2**, as illustrated below. However, there is no carbon in **2'** that would show a $^{3}J$ gHMBC correlation with both hydrogen atoms. Thus **2'** is excluded on the basis of gHMBC. Additionally, AM1 calculations yield a heat of formation of –46.3 kcal/mol for **2** and -30.9 (*trans*) and –26.8 kcal/mol (*cis*) for **2'** so **2** is highly favored under equilibrating conditions.




Conclusion.

NDA reacts with cyanide/methanol in the absence of amino acid to yield an air-oxidized condensation product **2**. The formation of **2** competes with formation of fluorescent isoindole in the NDA/cyanide/amino acid reaction in methanol. We have noted formation of **2** as a minor product by $^{1}H$ NMR when reacting aspartic acid with NDA/sodium cyanide in methanol. The key steps in formation of **2** appear to be (1) facile cyanide-catalyzed air oxidation of the NDA to either an acyl cyanide or methyl ester and (2) benzoin condensation. Exclusion of air was shown to avoid formation of **2**.

## EXPERIMENTAL

Reaction of Naphthalene-2,3-dicarbaldehyde (NDA) (**1**) with NaCN.

NDA (**1**) (35 mg, 0.22 mmol) was dissolved in 3.8 mL dry methanol, then NaCN (12.3 mg, 0.25 mmol) was added and the solution was reacted by shaking for 10 minutes. A yellow precipitate began to crystallize within 5 minutes. The solution was cooled for 10 minutes in an ice bath and centrifuged and the supernatant was removed. The precipitate was washed twice with cold water to remove excess sodium cyanide. The product was dried under vacuum and identified as 6-hydroxybenzo[*g*]benzo-[5,6]indeno[1,2-*c*]isochromen-14(6*H*)-one (**2**), 29.5% mp 287-289; IR (KBr): 3439 (OH) 1725 (doublet, O-C=O) 1624, 1279, 1155; $^{1}H$ NMR (300 MHz, DMSO-$d_6$): δ (ppm) 8.96 (s, 1H, H-8), 8.36 (s, 1H, H-13), 8.25 (d, 1H, 8.3 Hz, H-9), 8.20 (d, 1H, 8.3 Hz, H-12), 8.15 (s, 1H, H-5), 8.10 (dd, 8.3 Hz, 1.3 Hz, H-4), 8.00 (s, 1H, H-16), 7.98 (dd, 1H, 8.3 Hz, 1.3 Hz, H-1), 7.91 (d, 1H, 6.9 Hz, OH), 7.75 (ddt, 1H, 8.3 Hz, 7.0 Hz, 1.3 Hz, H-11), 7.65 (ddt, 8.3 Hz, 7.0 Hz, 1.3 Hz, H-10), 7.58 (ddt, 1H, 8.3 Hz, 7.0 Hz, 1.3 Hz, H-3), 7.55 (ddt, 1H, 8.3 Hz, 7.0 Hz, 1.3 Hz, H-2), 6.66 (d, 1H, 6.9 Hz, H-15); $^{13}C$ NMR (75 MHz, DMSO-$d_6$): δ (ppm) 160.7 (C-7), 135.8 (C-12a), 133.8, 132.7 (C-5b,C-16a), 133.4 (C-4a), 133.1 (C-8), 132.4 (C-8a), 130.9 (C-13b), 130.1 (C-11), 129.80 (C-9), 129.83 (C-15a), 128.5 (C-13a), 128.4 (C-4), 128.3 (C-12), 128.2 (C-1), 127.5 (C-10), 127.2 (C-3), 126,7 (C-2), 125.3 (C-16), 121.9 (C-5a), 119.9 (C-13), 119.0 (C-7a), 118.1 (C-5), 93.1(C-15); gHMBC, gCOSY, gHSQC, and NOESY spectra are available in the Supporting Information; Mass Spectrometry; LRFAB (3-NBA) *m/z* 349.1 ($M^+$-OH), 366.1 ($M^+$) (3-NBA/Li) 373.1 ($M^+$+Li).

*Anal*. Calcd. for $C_{24}H_{14}O_4$: C, 78.7; H, 3.85; O,17.5. Found: C, 78.9; H, 3.98; O 17.1.

15-Methoxybenzo[*g*]benzo[6,7]isochromeno[4,3-*c*]isochromen-7(15*H*)-one (**3a**).

Compound **2** (8.0 mg, 0.022 mmol) was dissolved in 3 mL methanol and 40 µL 6 *N* HCl was added. The reaction was refluxed for 1 hour and cooled to 0 °C. The precipitate was centrifuged, washed with cold water, dried and analyzed without further purification, 90% mp 220-222 °C; $^{1}H$ NMR (DMSO-$d_6$): δ (ppm) 9.02 (s, 1H, H-8), 8.52 (s, 1H, H-13), 8.29 (d, 1H, H-9), 8.26 (d, 1H, H-12), 8.23 (s, 1H, H-5), 8.16 (d,1H, H-4), 8.11 (s, 1H, H-16), 8.03 (d, 1H, H-1), 7.78 (dt, 1H, H-11), 7.70 (dt, 1H, H-10), 7.63 (dt, 1H, H-3), 7.59 (dt, 1H, H-2), 6.54 (s, 1H, H-15), 3.64 (s, 3H, $OCH_3$).

*Anal*. Calcd. for $C_{25}H_{16}O_4$ C: 78.9; H, 4.24. Found: C, 79.1; H, 4.21.

15-Isopropoxybenzo[*g*]benzo[6,7]isochromeno[4,3-*c*]isochromen-7(15*H*)-one (**3b**).

Compound **2** (5 mg, 0.014 mmol) was dissolved in 1 mL isopropanol and 3 drops of 6 *N* HCl was added. The reaction was refluxed for 10 minutes and and cooled to 0 °C. The precipitate was centrifuged, washed with cold water, dried and analyzed without further purification, 85%, mp 208-210 °C; $^{1}H$ NMR ($CDCl_3$): δ (ppm) 8.93 (s, 1H, H-8), 8.25 (s, 1H, H-13 or H-5), 8.23 (s, 1H, H-13 or H-5), 8.00 (d, 1H, H-9), 7.97 (d, 1H, H-12), 7.88 (d, 1H, H-4), 7.81 (d, 1H, H-1), 7.74 (s, 1H), 7.63 (dt, 1H, H-11), 7.53 (dt, 1H, H-10), 7.48 (dt, 1H, H-3), 7.44 (dt, 1H, H-2), 6.41 (s, 1H, H-15).

*Anal*. Calcd. for $C_{27}H_{20}O_4$ C: 79.4; H, 4.94. Found: C, 79.4; H, 4.89.

Acknowledgement.

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