SYNTHESIS OF NECTRIAFURONE, AN ISOFURANONAPHTHOQUINONE ISOLATED FROM THE FUNGUS NECTRIA HAEMATOCOCCA

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Abstract- Syntheses of 5-methoxy-4,7-dihydroxynaphtho[2,3-c]8,9-furandione (**2**) and of 4,7-dihydroxynaphtho[2,3-c]8,9-furandione (**3**) were carried out by a rapid Friedel-Crafts reaction using the dry method. The addition of acetaldehyde on **2** in presence of LDA gave the (±)-nectriafurone (**1**), thus confirming the proposed structure of 3-(1'-hydroxyethyl)-5-methoxy-4,7-dihydroxynaphtho[2,3-c]8,9-furandione.

Nectriafurone (**1**), the first described naturally occurring isofuranonaphthoquinone, was previously[1] isolated from cultures of the fungus Nectria haematococca (Berk. and Br.)Wr. This yellow, under uv strongly fluorescent pigment, was found together with pyranonaphthoquinones related to fusarubin. It was postulated to be a rearrangement product of some of these substances. However, all attempts carried out in order to convert anhydrofusarubin to **1** were unsuccessful. Later, differently substituted isofuranonaphthoquinones isolated[2] from Ventilago maderaspatana (Rhamnaceae) were reported. Friedel-Crafts reactions between 2- or 2,5-substituted furan-3,4-dicarboxylic acids (as their acid dichlorides) and appropriate phenolic compounds were shown[3-5] to give the corresponding isofuranonaphthoquinones. Authors mentioned that it was not possible to get 1,3-unsubstituted isofuranonaphthoquinones from the furan-3,4-dicarboxylic acid dichloride (**7**) by using this method. As a matter of fact, we were unable to get **2** or **3** by reacting the dichloride (**7**) on methoxyhydroquinone (**8**) or on hydroquinone (**9**) by reflux in nitrobenzene as reported. The expected isofuranonaphthoquinones (**2** or **3**) could be obtained by the similar Friedel-Crafts reaction carried out according to the rapid dry method[6] (no solvent). The commercially available diethyl 3,4-furandicarboxylate (**5**) was saponified to the diacid (**6**)

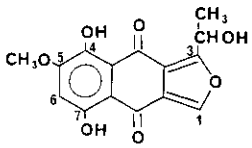
(70%) yield) which was converted into the corresponding dichloride (**7**) by reflux in oxalyl chloride. The Friedel-Crafts reaction between **7** and 2-methoxyhydroquinone (**8**) was found to occur when a mixture of $AlCl_3$ and NaCl was used and heated to 220°C, the reaction[6] being then immediately stopped. The resulting tar was submitted to the usual work up. The organic extract which contained the demethylated (**4**) was taken in an ether solution of diazomethane. The substance (**2**) was isolated from the reaction mixture by preparative SiO_2 tlc (18% yield). It exhibited a strong yellow fluorescence under uv (as the natural compound **1**). The ms and ^1H nmr spectra of **2** were in agreement with the expected structure. In particular, the two isofuranic protons were found at 8.20 and 8.25 ppm (while the corresponding H-atom of natural nectriafurone (**1**) was reported[1] at 8.10). The symmetrical analogue (**3**) was similarly obtained by a reaction between **7** and hydroquinone (**9**) (20% yield). In this case, the two isofuranic protons gave a single signal at 8.20. The structure (**2**) was in agreement with the proposed structure (**1**) for nectriafurone, particularly with regard to the chemical shift of the aromatic proton at C-6 (6.70 here and 6.66 in **1**) thus excluding[7] the alternative skeleton of an isomeric 4,7-naphthoquinone.

(±)-Nectriafurone (**1**) was obtained by the addition of acetaldehyde on the naphthoquinone (**2**) in presence of LDA (both reagents in large excess) in THF at -60°C in anhydrous conditions. Small amounts of the 1-substituted isomer (**10**) and of the 1,3-disubstituted product (**11**) were also detected. These 3 substances (**1**, **10** and **11**) were isolated by preparative SiO_2 tlc, the respective yields from **2** being 43%, 8%, 3%, or calculated from the starting diacid (**6**) : 8%, 1.5% and 0.5%.

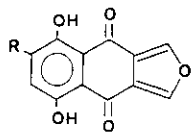
The direct comparison of the synthetic nectriafurone (**1**) with the natural compound previously isolated from cultures of the fungus Nectria haematococca confirmed the identity [Rf, mp 228-232°C, uv, ms, high resolution ms, ^1H nmr (signal of the isofuranic proton C-1 found at 8.10)]. Acetylation of **1** afforded an amorphous triacetate which gave the same fragmentation pattern in ms as reported.[1] The preferred addition of acetaldehyde at position 3 of **2** is obviously controlled by the electron donating effect of the methoxy group at C-5, leading to the deactivation of the farest carbonyl group and therefore favouring the metallation by LDA at C-3. This relative regiospecificity of the addition may also explain why the disubstituted compound (**11**) was only obtained in minute amounts although the reaction was carried out with a large excess of reagents. No substitution could be observed by using 2 to 6 times the theoretical amounts of LDA and acetaldehyde.

Rotatory power could not be determined for the natural nectriafurone[1] due to the minute amounts

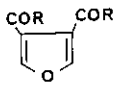
isolated from Nectria haematococca, so that the problem of the stereochemistry at the chiral C-atom is still pending and requires further experiments.



1



2 R = OCH_3
3 R = H
4 R = OH



5 R = OC_2H_5
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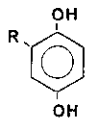
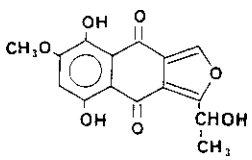


8 R = OCH_3
9 R = H



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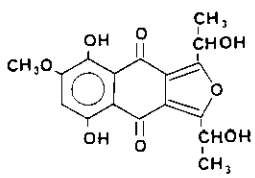

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The identity of the synthetic (1) with the natural nectriafurone is an argument in favour of the substitution occurring at C-3 to give the major product. Naphthoquinones in this series are known[1] to result from the cyclization of an heptaketide in which of course, the hydroxyethyl group is derived from the first acetate unit.

EXPERIMENTAL

Mps were determined on a Kofler apparatus under the microscope and are corrected, uv spectra were recorded on a Varian Lambda-5 spectrophotometer, ms were determined on an AEI MS 50 and ^1H nmr on Bruker 200 and 400 MHz spectrometers ($CDCl_3$, δ ppm, using TMS as internal standard).

5-Methoxy-4,7-dihydroxynaphtho[2,3-c]8,9-furandione (2). Diethyl 3,4-furandicarboxylate **5** (1 g, 4.7 mmol) was dissolved in ethanol (100 ml) and KOH (1 g, 17.8 mmol) was added. The saponification was carried out under reflux (ca. 30 min), the ethanol was concentrated of the 3/4th in vacuo, water (60 ml) was added, and the mixture was acidified to pH 1 (4M HCl) previous to extraction by ether (2 x 80 ml). The ether extracts were washed with H_2O, dried over Na_2SO_4. Evaporation of the solvent gave (**6**) as an amorphous white powder (515 mg, 70%) : mp 215-216°C as reported.[8] In order to prepare the corresponding dichloride (**7**), compound **6** (468 mg, 3 mmol) was dried overnight under vacuo. It was refluxed in oxalyl chloride (15 ml, 171 mmol) until completely dissolved and the excess of reagent was evaporated in vacuo. A mixture of 2-methoxyhydroquinone (**8**) (420 mg, 3 mmol), with freshly sublimed $AlCl_3$ (1.3 g, 9.7 mmol) and NaCl (300 mg, 5 mmol) was added to the dichloride (**7**) under stirring. The homogeneous mixture was then progressively heated to 220°C on an oil bath. After cooling at 0°C, water (100 ml) and 6 M HCl (60 ml) were added under stirring, the resulting suspension being kept overnight at room temperature.

Extraction with ethyl acetate (3 x 100 ml) gave a dark brown organic phase with a greenish fluorescence in uv (Desaga lamp, at 366 nm). The ethyl acetate extracts were washed with H_2O and dried over Na_2SO_4, leading to a dark residue (695 mg). This product was treated for 2 h with an excess of an ether solution of diazomethane at 0°C, after what an unsoluble part was discarded by filtration on a cotton plug. Control SiO_2 tlc ($CHCl_3$-MeOH 49:1) showed a single brilliant yellow substance at Rf 0.80 which was isolated by preparative tlc in the same solvent. A similar tlc performed previously to the diazomethane treatment had shown that demethylation had occurred at C-5 during the Friedel-Crafts reaction (presence of a more polar, tailing yellow substance at Rf 0.60 which revealed in ms to be **4**). Elution of substance (**2**) from the scrapped SiO_2 layer required a mixture of ethyl acetate and methanol (3:2) and a small amount more strongly chelated could be recovered with 1% methanolic HCl (total 120 mg, 18% yield from **6**). Analytical grade of **2** was prepared by a second tlc in the same conditions but carried out on Schleicher-Schüll films (SiO_2). The compound (**2**) was crystallized from ethyl acetate-pentane, giving orange-red microcrystals mp 262-266°C (formation of bigger needles before melting). The synthetic product (**2**), as the substances **1**, **3**, **4**, shows an intense yellow fluorescence in the higher part of the uv spectrum, which appears to be related to the isofuranonaphthoquinone ring system. Ms, m/z: 260 (M^+) 100%; ^1H nmr: 8.20 (s, 1H, C-1), 8.25 (s, 1H, C-3), 6.70 (s, 1H, C-6), 4.00 (s, 3H, OCH_3), 13.45 and 13.35 (s, 1H each, OH C-4 and C-7); uv (EtOH, nm, ε): 255 (9.4×10^4), 318 (2×10^3), 443 (4.6×10^3), 465 (3.5×10^3). Anal. Calcd for $C_{13}H_8O_6$: C, 60.01; H, 3.10. Found: C, 60.18; H, 3.35.

4,7-Dihydroxynaphtho[2,3-c]8,9-furandione (3). Hydroquinone (**9**) (110 mg, 1 mmol), $AlCl_3$ (450 mg, 3.6 mmol) and NaCl (100 mg, 1.7 mmol) were introduced into the acid dichloride (**7**) (prepared from 156 mg (**6**), 1 mmol). The mixture was progressively heated to 220°C as mentioned for substance (**2**). The work up of the resulting tar gave the compound (**3**) (47 mg, 20% yield). The product was crystallized from ethyl acetate-pentane, giving orange-brown microcrystals (30 mg, brilliant yellow fluorescence in uv as for (**2**), mp 223-226°C with formation of long needles before melting). Ms m/z: 230 (M^+) 100%; ^1H nmr: 7.30 (s, 2H, C-5 and C-6), 8.20 (s, 2H, C-1 and C-3), 12.80 (s, 2H, OH at C-4 and C-7). Anal. Calcd for $C_{12}H_6O_5$: C, 62.53; H, 2.63. Found: C, 62.65; H, 2.71.

(±)-Nectriafurone (1). Compound (**2**) (52 mg, 0.2 mmol) was dissolved in anhydrous THF (15 ml). The solution was kept at -60°C under stirring. An excess of LDA (8 ml) was introduced with a syringe through a septum. The LDA was prepared from THF (8 ml), diisopropylamine (880 mg, 8.7 mmol) (on molecular sieve) and butyllithium (4.8 ml, 11 mmol) of a 15% solution in hexane. Acetaldehyde (6 ml, 106 mmol) (on molecular sieve) was added, and after 5 min the flask was taken off from the cold bath and allowed to stay at room temperature for 5 min more. The pH of the mixture was brought to 2 by addition of 1M HCl. After addition of water (20 ml) the reaction mixture was extracted with ethyl acetate (2 x 50 ml). The organic phase was washed with H_2O, dried over Na_2SO_4 and evaporated. Tlc on SiO_2 ($CHCl_3$-MeOH 49:1) indicated 4 yellow substances at Rf 0.80 (**2**), 0.75 (**10**), 0.70 (**1**) and 0.60 (**11**). Preparative tlc gave the corresponding products, **2** (6 mg); **10** (4.8 mg, 8%); **1** (26 mg, 43%); **11** (2 mg, 3%). A second tlc gave pure **1** which was crystallized from ethyl acetate-pentane; yellow grains, mp 228-232°C rearrangement into small needles occurring from 210°C, (natural (**1**) mp 230°C); uv (EtOH, nm, ε): 255 (9.5 x 10^4), 443 (4.6 x 10^3), 465 (3.5 x 10^3); ms m/z: 304 (M^+) 100%; 289 ($M-15^+$) 43%; 286 ($M-18^+$) 42%; 261 ($M-43^+$) 64%; ^1H nmr (400 MHz): 1.65 (d, J=6 Hz, 3H, CH_3), 3.95 (s, 3H, OCH_3), 4.70 (d, J=6 Hz, 1H, OH), 5.20 (q, J=6 Hz, 1H, CH), 6.65 (s, 1H, C-6), 8.10 (s, 1H, C-1), 13.40 and 13.10 (phenolic protons C-4 C-7); high resolution ms, calcd for $C_{15}H_{12}O_7$: 304.05829, found: 304.0579. The triacetate of (**1**) was prepared by action of acetic anhydride in pyridine (1:1) overnight at room temperature. The product was recovered by direct evaporation at 20°C under high vacuo. Ms m/z 430 (M^+): 1%, 388 ($M-42^+$) 26%; 346 ($M-42-42^+$) 57%; 286 ($M-42-42-42^+$) 100%.

1-(1'-Hydroxyethyl)-5-methoxy-4,7-dihydronaphtho[2,3-c]8,9-furandione (10). This compound was crystallized from ethyl acetate-pentane, mp 184-188°C, ms m/z: 304 (M^+) 100%; high resolution ms, calcd for $C_{15}H_{12}O_7$ 304.05829, found: 304.0578; ^1H nmr *was identical to the above reported* spectrum[1] for **1** (as corresponding to the alternative addition product).

1,3-Di-(1'-hydroxyethyl)-5-methoxy-4,7-dihydroxynaphtho[2,3-c]8,9-furandione (11). This compound was obtained as an amorphous yellow powder which could not be crystallized; it decomposed (200-220°C) before melting; ms m/z: 348 (M^+) 45%, 305 ($M-43^+$), 55%, 304 ($M-44^+$), 100%, 289 ($M-44-15^+$), 50%, 287 45%, 286 40%, 273 66%, 262 55%; high resolution ms, calcd for $C_{17}H_{16}O_8$ 348.08451, found: 348.0845; ^1H nmr: 1.55 (d, 3H, CH_3), 1.65 (d, 3H, CH_3), 3.95 (s, 3H, OCH_3), 4.70 (s, 2H, OH), 5.20 (m, 2H, hydroxyethyl groups), 6.65 (s, 1H, C-6), 13.10 and 13.15 (OH phenolic groups); the coupling constants for the hydroxyethyl protons could not be assigned due to the overlapping of the signals.

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