SYNTHESIS OF NAPHTHOQUINOLES AS POTENTIAL ANTITUMOR AGENTS RELATED TO JACARANONE

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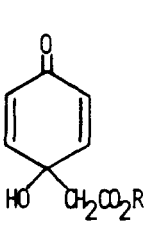
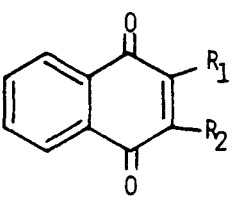
ABSTRACT.—Naphthoquinols **3a**, **3b** and **3c** were synthesized from the corresponding naphthoquinones by the series of reactions, protection of one carbonyl with trimethylsilycyanide, reaction with the enolate of methyl acetate, and hydrolytic deprotection with aqueous silver fluoride. The unusual internal hemiketals **4a** and **4b** appeared to be byproducts of one of these reactions. These naphthoquinols, which are related to the antitumor compound jacaranone (**1a**), were tested for cyctotoxicity and found to be inactive.

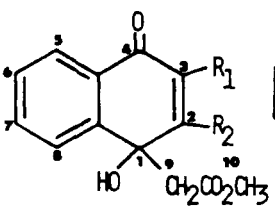
In 1976 Farnsworth *et al* (1) isolated the novel benzoquinol jacaranone (**1a**) from *Jacaranda caucana*. The aqueous methanolic extract of this plant had shown activity in the P-388 tumor system; fractionation of this extract led to the isolation of **1a** as the principle active compound (T/C = 165 at 2 mg/kg in P-388, ED_{50} = 2.1 μg/ml in KB). Since that time **1a** and the related ethyl ester **1b** have been isolated from other plants (2, 3).



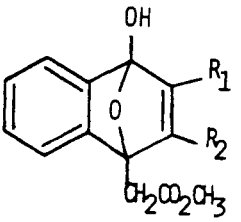
1a R = CH_3
1b R = CH_2CH_3



2a $R_1 = R_2 = H$
2b $R_1 = OCH_3, R_2 = H$
2c $R_1 = CH_3, R_2 = H$



3a $R_1 = R_2 = H$
3b $R_1 = OCH_3, R_2 = H$
3c $R_1 = CH_3, R_2 = H$
3d $R_1 = H, R_2 = CH_3$



4a $R_1 = CH_3, R_2 = H$
4b $R_1 = H, R_2 = CH_3$

Because of the relatively high biological activity of this simple benzoquinol we decided to synthesize several related naphthoquinols as potential antitumor agents. A synthetic approach utilizing the methodology of Evans (4, 5, 6) was envisioned. Thus, various naphthoquinones would have one of their carbonyl groups protected with trimethylsilyl cyanide, the enolate of methylacetate would be added to the other carbonyl, and hydrolytic deprotection would generate the naphthoquinol.

This approach was successful with naphthoquinones **2a**, **2b**, and **2c**. With **2a** only one isomer is possible (**3a**) and it was obtained in 85% crude yield, 64% after recrystallization. With **2b** only one isomer was obtained (**3b**) due to the strong directive effect of the methoxy group during the protection step (4). In this case the yield of crude product was only 59%, and it proved difficult to purify; the recrystallized yield was 41%. The structures of both **2a** and **2b** were completely consistent with their ^{1}H-nmr, ms, ir, uv spectra, as well as analytical data. With **2c** the situation was more complex as both isomers were apparently formed during the protection step. The crude product appeared to be two components by tlc and nmr and was chromatographed on silica. The more polar component (28%) crystallized and was identified as **3c** based on its ^{1}H-nmr, ms, ir, uv spectra, as well as analytical data. The faster eluting component (39%) was not obtained in pure form but, based on its nmr spectrum, appeared to be two closely related isomers similar to **3d**. After preparative hplc the two isomers could be partially separated but would not crystallize. Examination of the uv and ir spectral properties of the major isomer (λ max ~265 (ε 340), 215 nm (4900); ν max 1725

cm^{-1}) compared to **3c** (λ max 275 (ϵ 5300), 250 nm (8700); ν max 1725 and 1660 cm^{-1}) showed that the ketone carbonyl group was no longer present. Based on the fact that the ^{1}H-nmr appeared to be generally compatible with structure **3d**, and the ms showed a molecular ion at $m/z=246$, we propose structures **4a** and **4b** for these components. The driving force for the formation of this internal hemiketal is not clear as it would appear as though an increase in strain energy and loss of conjugation would result.

As shown in table 1, all of these compounds were screened for cyctotoxicity (7) in the KB *in vitro* test system. None of the compounds were active ($ED_{50}>4$ μg/ml), as compared to **1a** and **1b**, and therefore no *in vivo* testing was done.

Thus, although we attempted to extend the cyctotoxicity of simple benzoquinols, none of the naphthoquinols looked promising. Possibly, the requirements for activity in this γ-hydroxy-α,β-unsaturated enone system involve the lack of substitution at either α, β double bond and the most reactive system possible for conjugate addition, a mechanism proposed for the activity of α, β-unsaturated ketones and esters (8).

TABLE 1. *In vitro* testing of quinols.

Compound	9 KB ED_{50}-μg/ml
3a	20
3b	25
3c	100
4a and **4b** (mixture)	37
1a	2.1
1b	3.3

EXPERIMENTAL[1]

PREPARATION OF METHYL (1,4-DIHYDRO-4-OXO-1-HYDROXY-1-NAPHTHALENE) ACETATE (**3a**). EXAMPLE OF GENERAL PROCEDURE.—*Part A.* To a solution of 2.46 g (0.0156 mole) of 1,4-naphthoquinone in 15 ml of acetonitrile was added 1.85 g (0.0187 mole) of distilled trimethylsilylcyanide and 10 mg of triphenylphosphine. The mixture was stirred at room temperature for about 4 h.

Part B. A solution of lithium diisopropylamide was prepared from 1.58 g (0.0156 mole) of diisopropylamine and 0.0156 mole of *n*-butyllithium in 50 ml of dry tetrahydrofuran (THF). This solution was cooled to −78°, and 1.16 g (0.0156 mole) of methyl acetate dissolved in THF was slowly added and maintained at −78° for 2 h. The solution from Part A was evaporated to remove all solvent, dissolved in 5 ml THF, and then added very quickly to the above enolate solution. The solution immediately turned green and the cooling bath was removed. As it warmed the green gave way to a yellowish cast which became yellow-brown with a slight blue fluorescence around the top. When the solution had warmed to about 0°, 0.83 g (0.0156 mole) of NH_4Cl dissolved in the minimum amount of water was added; it became much darker. A portion of $MgSO_4$ was added, and the solution was filtered and evaporated *in vacuo*. The residue was dissolved in 25 ml of THF, and a solution of 2.65 g (0.0156 mole) of silver nitrate and 1.47 g (0.0156 mole) of potassium fluoride dihydrate in the minimum amount of water was added. The mixture was stirred for 2 h and a small amount of brine added. After filtering and washing the residue with 100 ml of ether, the solution was washed with saturated sodium bicarbonate, brine, dried over magnesium sulfate, filtered and evaporated; 3.1 g (85%) of brown solid was obtained. The solid was dissolved in 15 ml of warm ethyl acetate and filtered through a column of alumina. An initial bright yellow fraction (0.2 g) was discarded and the balance of the eluted material was recrystallized from ethyl acetate-hexane. This gave 2.3 g (64%) of **3a** as a very pale yellow solid, mp 127–128°; νmax ($CHCl_3$) 3560, 3450, 3025, 2990, 2940, 1720, and 1675 cm^{-1}; λmax (EtOH) 269 (ϵ 5800), 242 (6900) and 227 nm (7500); nmr ($CDCl_3$) δ 2.75 (1H, d, $J=15$ Hz, H-9), 3.02 (1 H, d, $J=15$ Hz, H-9), 3.63 (3 H, s, H-10), 4.5 (1 H, bs, OH), 6.30 (1 H, d, $J=10$ Hz, H-3), 7.20 (1 H, d, $J=10$ Hz, H-2), 7.4–8.2 (4 H, m, H-5, 6, 7, 8); mass spectrum m/z 232 (M^+, 1%), 200 (M^+-CH_3OH, 2), 159 ($M^+-CH_2CO_2CH_3$,

[1]Melting points were taken on a Thomas Hoover capillary apparatus and are uncorrected. Ir spectra were recorded with a Perkin Elmer 237B spectrophotometer. Nmr spectra were obtained with a Varian T60 spectrometer with Me_4Si as an internal standard ($\delta=0$). Ultraviolet determinations were done in 95% ethanol solvent on a Perkin Elmer 202 spectrophotometer. Mass spectra were run on a Hitachi RMU-7 spectrometer. Liquid chromatography was performed either with a Waters Assoc. Prep 500 or a Laboratory Data Control Corp. HPLC system. Analytical determinations were performed by Atlantic Microlabs, Inc., Atlanta, Ga.

100), and 131 (M^+–$COCH_2CO_2CH_3$, 22). *Anal.* Calcd. for $C_{13}H_{12}O_4$: C, 67.23; H, 5.21. Found: C, 67.18; H, 5.27.

PREPARATION OF METHYL (1,4-DIHYDRO-4-OXO-3-METHOXY-1-HYDROXY-1-NAPHTHALENE) ACETATE (**3b**).—The same general procedure as the preparation of **3a** was followed. Thus, the protected quinone (Part A) was prepared from 10.0 g (0.0532 mole) of 2-methoxynaphthoquinone and 5.79 (0.0585 mole) of trimethylsilylcyanide in 50 ml of acetonitrile in the presence of a catalytic amount of 18-crown-6/potassium cyanide complex. It was added to 0.0532 mole of the enolate from methyl acetate. After quenching with ammonium chloride and hydrolysis with silver fluoride in aqueous THF, the mixture was filtered and the THF distilled off. Ether and brine were added and the ether layer separated, dried and evaporated; 3.3 g (25%) of dark brown solid was obtained. Extraction of the aqueous solution with chloroform gave an additional 4.6 g (total—59%). This material was recrystallized from chloroform to give a light tan solid, mp 139–140°; νmax ($CHCl_3$) 3670, 3500, 2980, 2950, 1720, 1680, and 1640 cm^{-1}; λmax (EtOH) 288 (ε 5500), 255 (9300), 225 (6500), and 212 nm (7400); nmr ($CDCl_3$) δ 2.75 (1 H, d, J=15 Hz, H-9), 3.08 (1 H, d, J=15 Hz, H-9), 3.68 (3 H, s, H-10), 3.72 (3 H, s, CH_3O-3), 4.13 (1 H, s, OH), 6.13 (1 H, s, H-2), and 7.3–8.3 (4 H, m, H-5, 6, 7, 8); mass spectrum *m/z* 262 (M^+, 1%), 230 (M^+–CH_3OH, 19), 189 (M^+–$CH_2CO_2CH_3$, 100), and 161 (M^+–$COCH_2CO_2CH_3$, 43). Anal. Calcd. for $C_{14}H_{14}O_5$: C, 64.11, H, 5.38. Found: C, 64.40; H, 5.41.

PREPARATION OF METHYL (1,4-DIHYDRO-4-OXO-3-METHYL-1-HYDROXY-1-NAPHTHALENE) ACETATE (**3c**).—The same general procedure as the preparation of **3a** was followed. Thus, 10.0 g (0.0581 mole) of 2-methylnaphthoquinone was reacted with 6.33 g (0.0639 mole) of trimethylsilylcyanide in 25 ml of acetonitrile with triphenylphosphine as a catalyst. This was added to 0.0581 mole of the enolate from methyl acetate. After isolation, as in the preparation of **3b**, 16.1 g (113%) of red oil was obtained. Nmr and tlc (silica, $CHCl_3$) of the crude product showed a small amount of starting material and two well-resolved products. The tlc plates (with fluorescent indicator) were also visualized with iodine vapor; the high R_f component could only be detected about 0.5 h after the development and drying were complete. Preparative liquid chromatography on a silica column with chloroform-hexane (1:1) as solvent cleanly separated these components. The three fractions obtained (in order of elution) were identified by nmr as; 2-methylnaphthoquinone (0.9 g), the faster eluting component (5.6 g, 39%, see discussion and subsequent experimental), and **3c** (4.0 g, 28%). The last fraction crystallized and, when recrystallized from ether-hexane, gave 2.7 g (19%) of an off white solid. This compound (**3c**) showed mp 65–66°; νmax ($HCCl_3$) 3570, 3460, 3030, 2980, 2940, 1710, 1660, and 1640 cm^{-1}; λmax (EtOH) 275 (ε 5300), 250 (8700), 229 (8100), and 210 nm (7200); nmr ($CDCl_3$) δ 1.93 (3 H, d, J=1.4 Hz, CH_3-3), 2.69 (1 H, d, J=16 Hz, H-9), 2.98 (1 H, d, J=16 Hz, H-9), 3.60 (3 H, s, H-10), 4.33 (1 H, s, OH), 6.90 (1 H, q, J=1.4, H-2), and 7.2–8.1 (4 H, m, H-5, 6, 7, 8); mass spectrum *m/z* 246 (M^+, 1%), 214 (M^+–CH_3OH, 8), 173 (M^+–$CH_2CO_2CH_3$, 100), and 145 (M^+–$COCH_2CO_2CH_3$, 17). Anal. Calcd. for $C_{14}H_{14}O_4$: C, 68.28; H, 5.73. Found C, 68.27; H, 5.73.

The faster eluting component from the previous chromatography appeared to be a mixture (*ca.* 85/15) of closely related isomers (**4a**/**4b**) by nmr, which showed ($CDCl_3$) δ 2.13 (major isomer, d, J=1.5 Hz, CH_3-2 or 3), 2.18 (minor isomer, d, J=1.5 Hz, CH_3-2 or 3), 2.66 (1 H, d, J=16 Hz, H-9), 2.89 (1 H, d, J=16 Hz, H-9), 3.70 (major isomer, s, H-10), 3.74 (minor isomer, s, H-10), 4.22 (1 H, s, OH), 6.13 (major isomer, q, J=1.5 Hz, H-2 or 3), 6.23 (minor isomer, q, J=1.5 Hz, H-2 or 3), 7.4–7.9 (4 H, m, H-5, 6, 7, 8). A further chromatography of this material using a preparative hplc column (Whatman Magnum 9) and 25% hexane in chloroform gave partial separation of these isomers as judged by their nmr spectra. The major isomer was about 93% pure and showed νmax ($CHCl_3$) 3570, 3490, 3060, 2950, and 1725 cm^{-1}; λmax (EtOH) ~265 (ε 340), 215 nm (4900); mass spectrum *m/z* 246 (M^+, 2%), 214 (M^+–CH_3OH, 5), 173 (M^+–$CH_2CO_2CH_3$, 100), and 145 (M^+–$COCH_2CO_2CH_3$, 14).

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