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being recrystallized from $CHCl_3/CH_3OH/C_6H_6$ to give **8**: 0.12 g (25.9%); mp 194–195 °C; ^1H NMR (CD_3CN/D_2O) δ 3.2 (m, 2 H, CH_2), 3.95 (s, 2 H, CH_2), 4.85 (m, 1 H, CH), 6.7–7.8 (m, 8 H, Ar H); ^{13}C NMR (CD_3CN/D_2O) δ 37.1, 46.0, 60.1, 119.4, 125.3, 127.5, 129.5, 132.4, 133.9, 137.3, 138.5, 148.1, 149.7, 173.4, 176.9, 178.1. Anal. Calcd for $C_{18}H_{18}N_2O_6$ (mol wt 358.3): C, 60.3; H, 5.1; N, 7.8. Found: C, 60.7; H, 5.4; N, 7.5.

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Registry No. **1**, 86-51-1; **2**, 43087-79-2; **4**, 83949-20-6; DL-**5**, 83949-21-7; DL-**6**, 83949-22-8; DL-**7**, 83949-23-9; DL-**8**, 83949-24-0; $HO_2CCH_2NHCOPh$, 495-69-2; GlyOMe·HCl, 5680-79-5; EC 1.14.2.1, 9013-38-1.

Efficient Synthesis of Non-K-Region *trans*-Dihydro Diols of Polycyclic Aromatic Hydrocarbons from *o*-Quinones and Catechols

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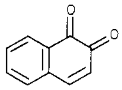
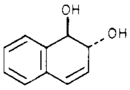
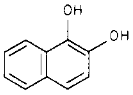
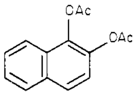
Non-K-region[1] dihydro diols of polycyclic aromatic hydrocarbons (PAH), generated by the epoxide hydrolase mediated hydration of metabolically formed arene oxides, play an important role in the metabolism of PAH.[2-7] They are precursors of dihydro diol epoxides, some of which are considered to be ultimate carcinogenic metabolites of PAH.[8-10]

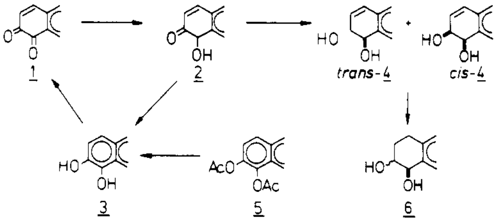
The two currently used methods for the synthesis of *trans*-dihydro diols are (a) the introduction of the *trans*-diol structure into a suitable dihydroarene by the Prévost reaction, followed by generation of the olefinic bond in the *trans*-dihydro diol either by dehydrogenation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone or by bromination–dehydrobromination, or (b) the reduction of a suitable *o*-quinone with complex metal hydrides. Non-K-region *trans*-dihydro diols have been prepared in most cases by pathway a[11-14] while pathway b has been almost exclusively used for the synthesis of K-region *trans*-dihydro diols.[15,16]

Table I. Reaction of 1,2-Naphthoquinone, 1,2-Dihydroxynaphthalene, and 1,2-Diacetoxynaphthalene with Sodium Borohydride

entry	starting matl	reaction conditions[a]	end product	yield, %
1	 **1a**	air, 48 h	 **4a**	80
2	**1a**	air, 5 min	 **3a**	82
3	**1a**	argon, 24 h	**3a**	82
4	**3a**	air, 48 h	**4a**	78
5	 **5a**	air, 18 h	**4a**	75

[a] Detailed reaction conditions are given in the Experimental Section.

Scheme I



In many instances, the application of pathway b for the synthesis of non-K-region dihydro diols would be desirable, either because of difficulties encountered when introducing the olefinic bond in the *trans*-dihydro diol or because the non-K-region *o*-quinone needed for pathway b can be prepared more conveniently than the dihydroarene needed for pathway a.

Attempts to reduce non-K-region *o*-quinones with lithium aluminum hydride started in 1950 when Booth et al.[17] successfully converted the 1,2-quinones of naphthalene and anthracene to the corresponding *trans*-dihydro diols in 46% and 25% yields, respectively. Application of this method to the synthesis of dihydro diols of other PAH, however, proved to be less successful as yields of 1–5% in the case of the non-K-region dihydro diols of phenanthrene[18] and of 15% in the case of *trans*-3,4-dihydroxy-3,4-dihydro-7-methylbenz[*a*]anthracene[19] illustrate. Recently it was reported that non-K-region *o*-quinones can be more efficiently reduced to dihydro diols with lithium aluminum hydride by improvements of the experimental conditions.[20-22] The yields of *trans*-dihydro diols according to these studies[20,21] vary between 0 and 61%.

The use of sodium borohydride for the reduction of non-K-region *o*-quinones of PAH to dihydro diols met with no success; this is illustrated by the 1.2% yield of *trans*-1,2-dihydroxy-1,2-dihydronaphthalene from 1,2-naphtho-

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quinone which was reported recently.[23] The efficient reduction of non-K-region *o*-quinones with sodium borohydride seemed hitherto only possible when the olefinic bond was first converted to the dibromide.[23,24]

Recently we observed that the presence of oxygen is indispensable for the successful conversion of K-region *o*-quinones to the corresponding dihydro diols with borohydrides.[16] We now find that also non-K-region *o*-quinones can be efficiently reduced to dihydro diols with sodium borohydride if the reaction is performed in the presence of oxygen.

Table I summarizes the results obtained when 1,2-dioxygenated derivatives of naphthalene are reduced with sodium borohydride. It should be emphasized that non-K-region catechols, e.g., **3a**, and their diacetates, e.g., **5a**, can conveniently replace the *o*-quinones, e.g., **1a**, all three compounds being converted to the dihydro diol, e.g., **4a**, if they are reduced with sodium borohydride in the presence of oxygen.

The role of oxygen is illustrated by the proposed reaction mechanism outlined in Scheme I. The non-K-region *o*-quinone **1** is reduced with sodium borohydride to the ketol **2** which isomerizes either to the fully aromatic catechol **3** or is further reduced to the dihydro diol **4**. In the case of 1,2-naphthoquinone (**1a**), aromatization of the ketol intermediate prevailed, and this was also the case with the polycyclic homologues of **1a**. Thus, after short reaction times (entry 2) and/or in an inert atmosphere (entry 3), catechol **3** is the main product, with only a small amount of dihydro diol **4** also being formed.

The final result of the reaction, however, depends on the fate of the primary reaction products **3** and **4**. Since the catechol is more easily reoxidized to the starting *o*-quinone than the dihydro diol, it can again be reduced, and the end product of this reduction–oxidation process in the presence of oxygen is pure dihydro diol. Further evidence for this reaction mechanism is the reduction of a catechol (**3a**, entry 4) and of catechol acetates, i.e., 1,2-diacetoxynaphthalene (**5a**, entry 5), 1,2-diacetoxyanthracene (**5b**), and 3,4-diacetoxydibenz[*a*,*h*]anthracene (**5l**), to the corresponding dihydro diols. In the latter cases, catechols are probably generated by reductive cleavage of the corresponding acetates with ethoxy borohydrides,[25] which are formed under the reaction conditions.

As has already been mentioned, the presence of oxygen is absolutely essential for the successful reduction of *o*-quinones to dihydro diols. In most cases, however, it is not necessary to pass oxygen through the reaction mixture, but the free access of air has to be ensured. The dihydro diols were usually obtained in good yield and were of high purity.

In some cases of bay-region[26] *o*-quinones, i.e., benz[*a*]anthracene-1,2-dione (**1e**), triphenylene-1,2-dione (**1h**) and benzo[*e*]pyrene-9,10-dione (**1k**), the primary formed catechols were rather stable to reoxidation by air. This allowed the isolation of the hitherto unknown catechol diacetates **5e**,**h**,**k** in good yields. Using pure oxygen and extending the reaction time to one week finally led to complete conversion of *o*-quinones **1h** and **1k** to dihydro diols which, however, suffered oxidative degradation, thereby reducing the yield. Benz[*a*]anthracene-1,2-dione (**1e**) was the only non-K-region *o*-quinone which could not be transformed to the dihydro diol since a longer reaction time under oxygen led to complete degradation. Generally we have noticed that non-K-region *trans*-dihydro diols in which the diol structure forms part of a bay region, e.g., *trans*-3,4-dihydroxy-3,4-dihydrophenanthrene (**4d**), are much more prone to oxidative degradation than their positional isomer at the same benzo ring, e.g., *trans*-1,2-dihydroxy-1,2-dihydrophenanthrene (**4c**). This difference in stability has been reported for a pair of non-K-region *trans*-dihydro diols of benzo[*a*]pyrene, **4i** and **4j**.[27]

Trans stereospecificity of the reduction was observed for all *o*-quinones in which the olefinic bond forms part of a bay region. Cis–trans mixtures of dihydro diols with the trans isomer being the main component were obtained in cases where the diketo structure of the *o*-quinone is located in the bay region, i.e., phenanthrene-3,4-dione (**1d**), triphenylene-1,2-dione (**1h**), and benzo[*a*]pyrene-9,10-dione (**1j**). This low degree of stereoselectivity is probably caused by steric interactions in the bay region, which could force the hydroxyl group of the ketol **2** (Scheme I) to adopt the axial conformation. Hydride transfer from the opposite, more accessible side of the molecule to the second carbonyl group in **2** would then lead to formation of the *cis*-dihydro diol. Similar results have been reported from reductions of K-region *o*-quinones with lithium aluminum hydride.[15]

Pure dihydro diols were usually obtained, but in one case, i.e., 1,2-dihydroxy-1,2-dihydrotriphenylene (**4h**), a significant amount of the tetrahydro diol **6h** was also formed. This can arise through further reduction of the initially formed dihydro diol, especially if longer reaction times are used.[21]

The results presented here demonstrate that non-K-region *o*-quinones and catechols can be efficiently and conveniently reduced to dihydro diols with sodium borohydride, in the presence of oxygen. Since the purity of the starting material seems to be crucial, we recommend the use of catechol diacetates **5** for the reductions since they can be prepared in a purer state and are more stable upon storage than *o*-quinones **1** and catechols **3**. Our method of preparing non-K-region *trans*-dihydro diols has the additional advantage of a very simple experimental procedure and workup and thus competes favorably with the laborious preparation using lithium aluminum hydride.

Experimental Section

Sodium borohydride was supplied by Merck. 1,2-Naphthoquinone was synthesized[28] since all commercially available samples of this quinone were found to be of unsatisfactory purity.

The NMR spectra were measured on a Varian EM 360 spectrometer at 60 MHz with Me_4Si as the internal standard. Mass spectra were obtained with a Varian CH7A mass spectrometer at 70 eV. UV absorption was measured with a Beckman 25 spectrophotometer. The HPLC separations were performed with a Spectra Physics SP 3500 B chromatograph. Melting points were determined in open capillary tubes and are uncorrected. All new compounds gave satisfactory microanalyses for C and H (within ±0.3%).

Reduction of 1,2-Naphthoquinone (1a). 1,2-Naphthoquinone (158 mg, 1 mmol) was added in small portions to a stirred suspension of sodium borohydride (494 mg, 13 mmol) in ethanol (50 mL), and the mixture was stirred under air at room temperature

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for 48 h. The resulting light colored suspension was poured into ice–water (100 mL), acidified with 2 N HCl, and extracted several times with CH_2Cl_2. The organic phase was washed neutral, dried ($MgSO_4$), and evaporated to dryness. Recrystallization from benzene yielded *trans*-dihydro diol **4a**: 130 mg (80%); white needles; mp 103 °C (lit.[17] mp 103 °C); NMR (acetone-d_6/D_2O) δ 4.25–4.53 (m, 1 H_2), 4.74 (d, 1, H_1, $J_{1,2}$ = 10.5 Hz), 5.95 (dd, 1, H_3), 6.38 (dd, 1, H_4), 6.93–7.63 (m, 4, aromatic).

The same reaction performed under air for 5 min or under argon for 24 h furnished, after recrystallization from hexane, catechol **3a**: 131 mg (82%); pink crystalline solid; mp 101–102 °C (lit.[29] mp 103–104 °C); MS, *m/e* (relative intensity) 160 (100, M^+), 131 (47), 102 (7).

The following reductions with sodium borohydride were performed as described for the preparation of **4a**.

Reduction of 1,2-Dihydroxynaphthalene (3a). Reaction of **3a** (160 mg, 1 mmol) with $NaBH_4$ (500 mg) in ethanol (50 mL) under air for 48 h yielded, after recrystallization from benzene, *trans*-dihydro diol **4a**, 126 mg (78%).

Reduction of 1,2-Diacetoxynaphthalene (5a). Reaction of **5a** (1.95 g, 8 mmol) with $NaBH_4$ (3.2 g) in ethanol (250 mL) under oxygen for 7 h yielded, after recrystallization from benzene, *trans*-dihydro diol **4a**, 1.08 g (83%).

Reduction of 1,2-Anthraquinone (1b). Reaction of **1b**[30] (104 mg, 0.5 mmol) with $NaBH_4$ (200 mg) in ethanol (15 mL) under oxygen for 20 h yielded *trans*-dihydro diol **4b**: 76 mg (72%); off-white solid; mp 177 °C (lit.[17] mp 170 °C); NMR (Me_2SO-d_6/D_2O) δ 4.26–4.54 (m, 1, H_2), 4.89 (d, 1, H_1, $J_{1,2}$ = 10.0 Hz), 6.00 (dd, 1, H_3), 6.60 (d, 1, H_4), 7.32–7.93 (m, 6, aromatic).

Reduction of 1,2-Diacetoxyanthracene (5b). Reaction of **5b**[31] (88 mg, 0.3 mmol) with $NaBH_4$ (200 mg) in 87% ethanol (15 mL) under air for 24 h yielded, after recrystallization from acetone, *trans*-dihydro diol **4b**: 51 mg (80%); white crystalline solid.

Reduction of Phenanthrene-1,2-dione (1c). Reaction of **1c**[32] (104 mg, 0.5 mmol) with $NaBH_4$ (200 mg) in 87% ethanol (15 mL) under air for 72 h yielded, after recrystallization from acetone–hexane, *trans*-dihydro diol **4c**: 69 mg (65%); white crystalline solid; mp 163 °C (lit.[12] mp 164–165 °C); NMR (acetone-d_6/D_2O) δ 4.37–4.63 (m, 1, H_2), 4.90 (d, 1, H_1, $J_{1,2}$ = 12.0 Hz), 6.17 (dd, 1, H_3), 7.17 (dd, 1, H_4), 7.28–7.90 (m, 5, aromatic), 8.00–8.23 (m, 1, H_5).

Reduction of Phenanthrene-3,4-dione (1d). Reaction of **1d**[32] (104 mg, 0.5 mmol) with $NaBH_4$ (200 mg) in 87% ethanol (15 mL) under air for 72 h yielded a product which consisted of a mixture of 9% *cis*-**4d** and 91% *trans*-**4d** according to TLC and UV spectrometry.[33] Preparative HPLC (RP-18; MeCN-H_2O, 46/54 v/v) furnished *trans*-dihydro diol **4d**: 58 mg (55%); white solid; mp 131–133 °C; NMR (acetone-d_6/D_2O) δ 4.29 (dd, 1, H_3, $J_{3,4}$ = 2 Hz), 5.27 (br s, 1, H_4), 6.10 (dd, 1, H_2), 6.61 (d, 1, H_1), 7.10–7.85 (m, 5, aromatic), 7.93–8.18 (m, 1, H_5).

Reduction of Benz[*a*]anthracene-1,2-dione (1e). Reaction of **1e**[32] (77 mg, 0.3 mmol) with $NaBH_4$ (110 mg) in ethanol (15 mL) under oxygen for 6 h afforded an air-sensitive product which was acetylated (Ac_2O, pyridine) and recrystallized from $CHCl_3$–EtOH, yielding catechol diacetate **5e**: 77 mg (75%); pale yellow solid; mp 183–185 °C; NMR ($CDCl_3$) δ 2.33 (s, 3, acetate), 2.57 (s, 3, acetate), 7.27–8.10 (m, 8, aromatic), 8.20 (s, 1, H_7), 9.47 (s, 1, H_{12}); MS, *m/e* (relative intensity) 344 (29, M^+), 302 (30), 260 (100), 231 (23), 202 (27).

The same reaction performed under oxygen for 48 h resulted in an inseparable mixture of degradation products.

Reduction of Benz[*a*]anthracene-3,4-dione (1f). Reaction of **1f**[21] (77 mg, 0.3 mmol) with $NaBH_4$ (200 mg) in ethanol (15 mL) under oxygen for 48 h yielded, after recrystallization from acetone, *trans*-dihydro diol **4f**: 55 mg (70%); white crystalline solid, mp 212–214 °C (lit.[12] mp 215–217 °C); NMR (Me_2SO-d_6/D_2O) δ 4.40–4.66 (m, 1, H_3), 4.91 (d, 1, H_4, $J_{3,4}$ = 11.0 Hz), 6.19 (dd, 1, H_2), 7.25–8.21 (m, 7, H_1 and aromatic), 8.41 (s, 1, H_7), 8.79 (s, 1, H_{12}).

Reduction of Benz[*a*]anthracene-8,9-dione (1g). Reaction of **1g**[32] (77 mg, 0.3 mmol) with $NaBH_4$ (150 mg) in 87% ethanol (17 mL) under air for 48 h yielded, after recrystallization from acetone, *trans*-dihydro diol **4g**: 54 mg (69%); white crystalline solid; mp 180–182 °C (lit.[12] mp 168–170 °C); NMR (Me_2SO-d_6/D_2O) δ 4.33–4.57 (m, 1, H_9), 4.88 (d, 1, H_8, $J_{8,9}$ = 10.0 Hz), 6.03 (dd, 1, H_{10}), 6.67 (d, 1, H_{11}), 7.53–8.17 (m, 6, aromatic), 8.47 (s, 1, H_{12}), 8.67–8.87 (m, 1, H_1).

Reduction of Triphenylene-1,2-dione (1h). Reaction of **1h**[32] (129 mg, 0.5 mmol) with $NaBH_4$ (200 mg) in 87% ethanol (15 mL) under air for 6 h afforded an air-sensitive product which was acetylated (Ac_2O, pyridine) and recrystallized from $CHCl_3$–EtOH, yielding catechol diacetate **5h**: 115 mg (67%); off-white crystalline solid; mp 145–146 °C; NMR ($CDCl_3$) δ 2.35 (s, 3, acetate), 2.45 (s, 3, acetate), 7.40 (d, 1, H_3), 7.50–7.71 (m, 4, $H_{6,7,10,11}$), 8.35–8.65 (m, 4, $H_{4,5,8,9}$), 8.85–9.07 (m, 1, H_{12}); MS, *m/e* (relative intensity) 344 (15, M^+), 302 (15), 260 (100), 231 (18), 202 (22).

The same reaction performed under oxygen for 1 week resulted in a mixture of 26% *cis*-**4h**, 43% *trans*-**4h**, and 31% tetrahydro diol **6h**. Preparative HPLC (RP-18; MeCN-H_2O, 55/45 v/v) furnished *trans*-dihydro diol **4h**: 26 mg (20%); white solid; mp 150–152 °C (lit.[34] mp 153–154 °C); NMR (acetone-d_6/D_2O) δ 4.52–4.74 (m, 1, H_2), 5.20 (d, 1, H_1, $J_{1,2}$ = 2 Hz), 6.41 (dd, 1, H_3), 7.51 (d, 1, H_4), 7.70–8.48 (m, 6, aromatic), 8.78–9.02 (m, 2, $H_{8,9}$).

Reduction of Benzo[*a*]pyrene-7,8-dione (1i). Reaction of **1i**[32] (141 mg, 0.5 mmol) with $NaBH_4$ (200 mg) in ethanol (20 mL) under oxygen for 3 h yielded, after recrystallization from acetone, *trans*-dihydro diol **4i**: 112 mg (78%); off-white platelets; mp 218 °C (lit.[11] mp 216–217 °C); NMR (Me_2SO-d_6/D_2O) δ 4.33–4.63 (m, 1, H_8), 5.00 (d, 1, H_7, $J_{7,8}$ = 10.6 Hz), 6.23 (dd, 1, H_3), 7.42 (dd, 1, H_4), 7.87–8.49 (m, 8, aromatic).

Reduction of Benzo[*a*]pyrene-9,10-dione (1j). Reaction of **1j**[32] (141 mg, 0.5 mmol) with $NaBH_4$ (250 mg) in 87% ethanol (25 mL) under air for 15 h yielded a product which consisted of a mixture of 22% *cis*-**4j** and 78% *trans*-**4j** according to TLC and UV spectrometry.[33] Preparative HPLC (RP-18; MeCN-H_2O, 46/54 v/v) furnished *trans*-dihydro diol **4j**: 95 mg (66%); white powder; mp 205–207 °C (lit.[27] mp 209–210 °C); NMR (acetone-d_6/D_2O) 4.42–4.64 (m, 1, H_9), 5.71 (d, 1, H_{10}, $J_{9,10}$ = 2 Hz), 6.38 (dd, 1, H_8), 7.02 (d, 1, H_7), 7.98–8.42 (m, 7, aromatic), 8.59 (d, 1, H_{11}).

Reduction of Benzo[*e*]pyrene-9,10-dione (1k). Reaction of **1k**[32] (85 mg, 0.3 mmol) with $NaBH_4$ (150 mg) in 87% ethanol (15 mL) under air for 21 h afforded an air-sensitive product which was acetylated (Ac_2O, pyridine) and recrystallized from $CHCl_3$–EtOH, yielding catechol diacetate **5k**: 71 mg (64%); pale yellow solid; mp 171 %C; NMR ($CDCl_3$) δ 2.37 (s, 3, acetate), 2.49 (s, 3, acetate), 7.50 (d, 1, H_{11}), 7.75–8.14 (m, 6, aromatic), 8.52–8.77 (m, 2, $H_{1,12}$), 9.23 (dd, 1, H_8); MS, *m/e* (relative intensity) 368 (47, M^+), 326 (44), 284 (100), 255 (22), 226 (33).

The same reaction was performed under oxygen for 1 week. The crude reaction product was acetylated (Ac_2O, pyridine) and purified by preparative TLC (SiO_2, $CHCl_3$). Cleavage of the diacetate (THF–MeOH, NaOMe) yielded *trans*-dihydro diol **4k**: 30 mg (35%); off-white powder; mp 182–184 °C (lit.[35] mp 185–186 °C); NMR (acetone-d_6/D_2O) δ 4.52 (dd, 1, H_{10}, $J_{9,10}$ = 2 Hz), 5.55 (br s, H_9), 6.56 (dd, 1, H_{11}), 7.68 (d, 1, H_{12}), 7.87–8.57 (m, 8, aromatic).

Reduction of Dibenz[*a,h*]anthracene-3,4-dione (1l). Reaction of **1l**[32] (92 mg, 0.3 mmol) with $NaBH_4$ (200 mg) in ethanol (30 mL) was performed under air for 48 h. The reaction product was acetylated (Ac_2O, pyridine) and recrystallized from acetone. Cleavage of the diacetate (THF–MeOH, NaOMe) yielded *trans*-dihydro diol **4l**: 67 mg (72%); pale yellow solid; mp 273–275 °C (lit.[23] mp 274–275 °C); NMR (Me_2SO-d_6/D_2O) δ 4.25–4.63 (m, 1, H_3), 4.88 (d, 1, H_4, $J_{3,4}$ = 10 Hz), 6.20 (d, 1, H_2), 7.20–8.31 (m, 8, H_1 and aromatic), 8.76–9.00 (m, 1, H_8), 8.84 (s, 1, H_{14}), 9.31 (s, 1, H_7).

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Reduction of 3,4-Diacetoxydibenz[*a*,*h*]anthracene (5l). Reaction of 5l[36] (79 mg, 0.2 mmol) with $NaBH_4$ (200 mg) in ethanol (15 mL) under air for 70 h yielded after recrystallization from THF *trans*-dihydro diol 4l, 44 mg (70%).

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Registry No. **1a**, 524-42-5; **1b**, 655-04-9; **1c**, 573-12-6; **1d**, 4733-11-3; **1e**, 82120-27-2; **1f**, 74877-25-1; **1g**, 82120-26-1; **1h**, 82120-28-3; **1i**, 65199-11-3; **1j**, 82120-25-0; **1k**, 82120-24-9; **1l**, 70644-35-8; **3a**, 574-00-5; **4a**, 771-16-4; **4b**, 4841-37-6; **4c**, 60917-41-1; *cis*-**4d**, 61616-82-8; *trans*-**4d**, 569-20-0; **4f**, 60967-89-7; **4g**, 34501-24-1; *cis*-**4h**, 82120-22-7; *trans*-**4h**, 68151-04-2; **4i**, 57404-88-3; *cis*-**4j**, 56484-47-0; *trans*-**4j**, 58886-98-9; **4k**, 66788-06-5; **4l**, 66267-19-4; **5a**, 6336-79-4; **5b**, 74877-26-2; **5e**, 84040-73-3; **5h**, 84040-74-4; **5k**, 84040-75-5; **5l**, 70644-34-7.

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Microminutin, a Novel Cytotoxic Coumarin from *Micromelum minutum* (Rutaceae)

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Introduction

Previous studies of *Micromelum* species, family Rutaceae,[1–4] have revealed a number of unique coumarins, e.g., micromelin (1, Chart I) in which an α,β-unsaturated γ-lactone is bound to a coumarin nucleus. Cassady et al.[3] have recently reported that 1 displays in vivo activity in the P-388 lymphocytic leukemia test system.[5]

The present study is concerned with the isolation and structure elucidation of microminutin (**2**), a novel cytotoxic coumarin from the leaves of *M. minutum* (Forst. f.) Seem. (syn. *Micromelum pubescens* Blume), collected in Thailand. The pyranoquinoline alkaloid flindersine **3** was also isolated in the course of these studies. Microminutin (**2**) was isolated in almost 1% yield from the plant, and its structure was determined from the following spectroscopic studies.

Discussion

The molecular formula $C_{15}H_{12}O_6$ indicated for the isolate was established by high-resolution mass spectrometry. The infrared spectrum showed a very strong, wide carbonyl band at 1740 cm^{-1} and suggested the presence of two unsaturated lactones or of one unsaturated lactone and one saturated ester group, an ambiguity that was resolved by NMR analysis. The IR spectrum did, however, indicate an absence of hydroxyl groups.

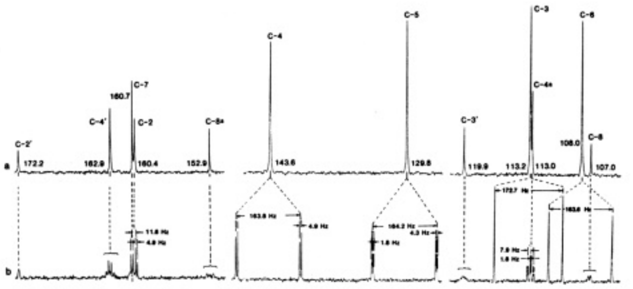

Figure 1. Carbon-13 NMR spectrum (25.05 MHz) of microminutin (**2**): (a) proton noise decoupled (PND); (b) coupled spectrum.

From previous studies[1–4] a coumarin nucleus was suspected for microminutin. This and the location and nature of the substituents on the nucleus were deduced by a combination of ^1H and ^{13}C NMR spectroscopy.

The proton noise decoupled (PND) ^{13}C spectrum of microminutin as well as the coupled ^{13}C spectrum (Figure 1) exhibit resonances of δ 160.4 as a doublet of doublets (J = 4.6, 11.4 Hz), δ 113.2 as a doublet (J = 172.6 Hz), and δ 143.6 as a doublet of doublets (J = 4.9, 163.0 Hz). These three resonance patterns are characteristic[6] of C-2, C-3, and C-4, respectively, of the coumarin nucleus.

The aromatic region of the ^1H NMR spectrum of microminutin at 360 MHz contains only four resonance patterns, all of which are doublets. A pair of doublets (9.5 Hz) at δ 6.25 and 7.68 are characteristic of H-3 and H-4 in a coumarin nucleus.[7] Irradiation of the H-4 doublet at δ 7.68 produces a positive nuclear Overhauser effect (NOE) in the doublet (J = 8.8 Hz) at δ 7.50, which can therefore be assigned to H-5, leaving the doublet at δ 6.93 as H-6. The absence of any other ^1H signals in the aromatic region clearly indicates that positions C-7 and C-8 are substituted and furthermore that their substituents do not possess any aromatic or olefinic protons.

The three-proton singlet at δ 3.90 may be assigned to either aromatic methoxy (OMe) or carbomethoxy (COOMe) protons, and this ambiguity could also be resolved by performing a double resonance experiment. Irradiation at the frequency of this signal shows a strong, positive (23.4%) NOE in the H-6 doublet at δ 6.93. This experiment suggests that the resonance at δ 3.90 should be the protons of a methoxy group located at C-7. Similar irradiation of a resonance from the protons of either a COOMe group at C-7 or a methoxy group at C-8 would not be expected to produce the above effect.

With the coumarin nucleus and its substitution pattern firmly established, attention was turned to the molecular array attached at C-8 in microminutin. From the high-resolution mass spectrum this fragment should have the molecular formula $C_5H_5O_2$ and the nature of this moiety was deduced through total assignment of the ^{13}C and ^1H NMR spectra. As an initial step, the carbon resonances representing the coumarin nucleus and the C-7 methoxy group were assigned by comparison of the ^{13}C spectra of

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