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Synthesis and antitubercular activity of tricyclic analogs of puupehenone

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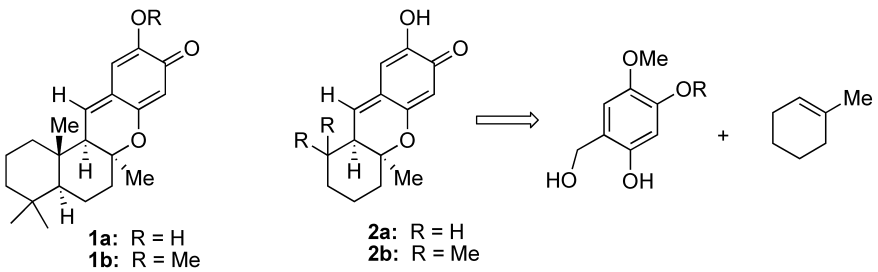
Abstract—Tricyclic analogs **2a** and **8** were prepared by four-step routes. The key step was an intermolecular hetero Diels–Alder reaction involving a quinone methide.

Puupehenone (**1a**), a tetracyclic terpene bearing a quinone methide subunit, was isolated from a deep water marine sponge, *Strongylophora hartmani*.[1] It exhibits 99% inhibition of *M. tuberculosis* $H_{37}Rv$ at 12.5 μg/ml.[2] Recently, *O*-methyl puupehenone (**1b**) was isolated and found to inhibit lipoxygenase activity at the sub micromolar level.[3] As part of a program to identify useful antitubercular agents for animal and human use,[4] we decided to synthesize and evaluate **2a**, a tricyclic analog of **1a**. Recently, a tricyclic *ortho*-quinone related to **2b** was synthesized by a route very different from our pathway and was a more active antitumor agent than **1a** (Scheme 1).[5]

We envisioned the synthesis of **2a** via a hetero-Diels–Alder reaction involving a quinone methide. Quinone methides were initially employed by Buchi in his elegant synthesis of gymnitrol.[6] The intramolecular version was used by Tius in his innovative synthesis of canniboid natural products.[7] Recently, Pettus and co-workers reported an interesting alkylative variant.[8] Quinone methides have been used in the synthesis of a number of compounds.[9]

Our route began with commercially available 3,4-dimethoxyphenol (**3**). Hydroxymethylation of phenols under basic conditions provides 2-hydroxymethylphenols.[10] When 3,4-dimethoxyphenol was treated with formalin and aqueous calcium oxide, the desired alcohol **4** was produced. Alcohol **4** was unstable to silica gel chromatography and decomposed in hours at ambient temperature. In practice, freshly prepared **4** was added to 1-methylcyclohexene and treated with trifluoroacetic acid using the protocol of Pettus[8] to afford the tricyclic benzopyran **5** in 45% yield over two steps from 3,4-dimethoxyphenol. Compound **5** was produced as a single regioisomer. The [4+2] cycloaddition generated both the carbon–carbon and carbon–oxygen bonds with a *cis* ring juncture, as determined by a NOESY experiment. The demethylation of **5** with boron tribromide afforded a catechol in 60% yield. Previous syntheses of puupehenone utilized a variety of oxidants to generate the quinone methide moiety. The reaction of PDC afforded an inseparable mixture of the desired quinone methide **2a** plus some of the *ortho*-quinone.



Scheme 1.

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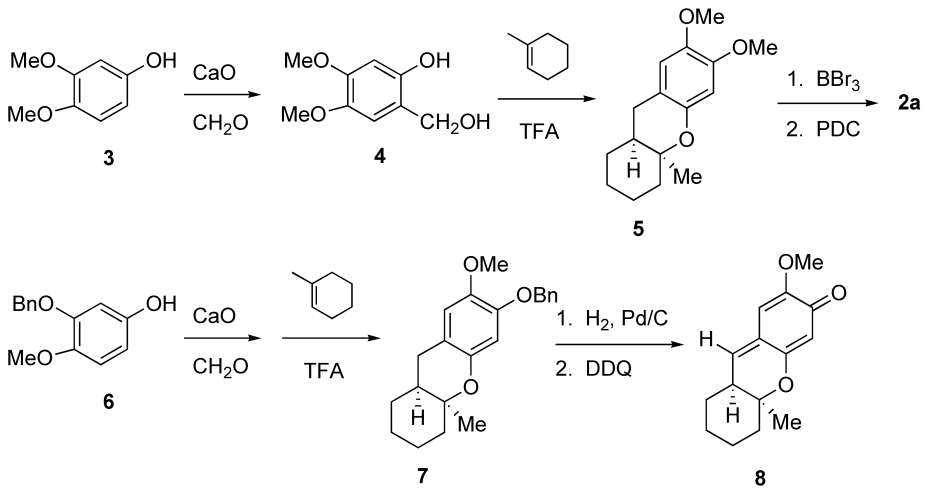

Scheme 2.

The synthesis of the *O*-methyl analog was also achieved. This synthesis began with the benzylation of isovanillin followed by oxidation of the aldehyde to a formate and hydrolysis of the formate with aqueous sodium hydroxide, to produce the known phenol **6** in 80% yield.[11] Hydroxymethylation of **6** with formalin and calcium oxide followed by a hetero-Diels–Alder reaction afforded benzopyran **7** in 40% yield over two steps. Catalytic hydrogenation[12] of compound **7** followed by treatment with DDQ, afforded product **8** in 95% isolated yield. The structure of **8** was confirmed by ^{1}H NMR, ^{13}C NMR, HRMS, and UV spectrometry (Scheme 2).

The combination of regioselective hydroxymethylation with the hetero-Diels–Alder reaction provides a convenient synthesis of tricyclic skeletons common to bioactive terpenes. This pathway should be compatible with considerable structural variation on both the phenol and alkene units.

Compound **8** and the aminoglycoside antibiotic gentamycin were tested in broth cultures of *Mycobacterium avium* subspecies *paratuberculosis* (*M. a. ptb*). Broth were sampled weekly and number of colony forming units were determined for each time point. Values are expressed as the percentage of the control treatment (bacteria in media alone). Preliminary results indicated that with a concentration of **8** at 0.625 μg/ml, after one week 43% of the bacteria remained, compared to 0.1% remaining with gentamycin.

1. Experimental

1.1. General

1.1.1. 2-Hydroxymethyl-4,5-dimethoxy-phenol (4). To 3,4-dimethoxyphenol (1.00 g, 6.5 mmol) in 20 mL of water at rt, was added 37% formalin (1.4 mL, 13.4 mmol) and followed by calcium oxide (0.18 g, 3.3 mmol). After one h, saturated aqueous ammonium chloride was added and the organic layer was extracted with ether, dried, concentrated and used immediately without purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.54 (s, 1H), 6.51 (s, 1H), 4.80 (s, 2H), 3.84 (s, 3H), 3.80 (s, 3H); ^{13}C NMR 159.9, 149.5, 142.3, 116.7, 112.5, 101.4, 62.8, 56.8, 55.9. TLC (2:1 hexanes/ethyl acetate) R_f=0.23.

1.1.2. 6,7-Dimethoxy-4a-methyl-2,3,4,4a,9,9a-hexahydro-1*H*-xanthene (5). To compound **4** (0.6 g, 3.26 mmol) in 40 mL of $CHCl_3$ at 0 °C, was added 1-methylcyclohexene (0.47 g, 4.9 mmol) followed by dropwise addition of trifluoroacetic acid (0.45 g, 3.91 mmol). The mixture was boiled for 3 h. After cooling to rt, the solution was diluted with water and extracted with CH_2Cl_2, dried, concentrated and purified by sgc using 4:1 hexanes/ethyl acetate to afford benzopyran **5** in 45% yield from 3,4-dimethoxyphenol. The *cis*-ring juncture was assigned by NOESY spectroscopy. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.52 (s, 1H), 6.38 (s, 1H), 3.82 (s, 3H), 3.81 (s, 3H), 3.03 (dd, J=12, 6 Hz, 1H), 2.25 (d, J=18 Hz, 1H), 1.94–1.90 (m, 1H), 1.67–1.24 (m, 8H), 1.19 (s, 3H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 148.4, 146.9, 142.8, 112.8, 110.31 101.3, 74.9, 56.6, 56.0, 38.7, 37.2, 29.2, 28.7, 25.8, 25.6, 21.9. HRMS (EI) calcd, for 262.1569, found 262.1572. TLC (2:1 hexanes/ethyl acetate) R_f=0.72.

1.1.3. 2-Hydroxy-10a-methyl-5,6,7,8,8a,10a-hexahydroxanthen-3-one (2a). To compound **5** (0.05 g, 0.19 mmol) in 5 mL of CH_2Cl_2 at −78 °C, was added slowly 1.0 M boron tribromide (0.57 mL, 0.57 mmol). After 30 min at −78 °C, the mixture was warmed to rt and stirred for 4 h. The solution was diluted with CH_2Cl_2 and water was added slowly. The aqueous layer was extracted with CH_2Cl_2 and the combined organic layers were washed with saturated $NaHCO_3$, brine, dried, concentrated and purified by sgc using 2:1 hexanes/ethyl acetate. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.69 (s, 1H), 6.37 (s, 1H), 3.00 (dd, J=15, 6 Hz, 1H), 2.21 (d, J=18 Hz, 1H), 1.91–1.10 (m, 9H), 1.21 (s, 3H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 147.2, 142.9, 137.0, 116.2, 111.9, 104.3, 74.9, 38.7, 37.1, 29.1, 28.6, 25.8, 25.6, 21.9; HRMS (EI) m/z calcd for 234.1256, found 234.1259. TLC (2:1 hexanes/ethyl acetate) R_f=0.37.

To the catechol (0.020 g, 0.085 mmol) in 2 mL of CH_2Cl_2 at rt was added PDC (0.05 g, 0.13 mmol). After 4 h, the mixture was filtered through Celite and concentrated to afford **2a** and the *ortho*-quinone.

Spectra for *ortho*-quinone. ^{1}H NMR (300 MHz) 6.17(dd, J=2.1, 0.9 Hz, 1H), 5.84(s, 1H), 3.09–3.16(m, 1H), 2.43(d, J=13.8 Hz, 1H), 1.96–2.04 (m, 1H), 1.43–1.89 (m, 1H), 1.40 (s, 3H).

Spectra for **2a**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.21 (d, J=1.5 Hz, 1H), 6.35 (s, 1H), 5.95 (d, J=3 Hz, 1H), 2.18–2.10 (m, 1H), 1.98–1.21 (m, 8H), 1.19 (s, 3H); HRMS (EI) m/z calcd for 232.1010, found 232.1102. TLC (2:1 hexanes/ethyl acetate) R_f=0.22.

1.1.4. 6-Benzyloxy-7-methoxy-4a-methyl-2,3,4,4a,9,9a-hexahydro-1*H*-xanthene (7). To compound **6** (0.8 g, 3.5 mmol) in 20 mL of water at rt was added 37% formalin (0.77 g, 7.35 mmol) followed by calcium oxide (0.098 g, 1.75 mmol). After an hour, saturated aqueous ammonium chloride was added and the organic layer was extracted with ether, dried, concentrated and used immediately without purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43–7.30 (m, 5H), 6.56 (s, 1H), 6.51 (s, 1H), 5.10 (s, 2H), 4.76 (s, 2H), 3.82 (s, 3H); ^{13}C NMR 149.2, 148.9, 142.9, 136.9, 128.8, 128.2, 127.7, 117.3, 113.4, 103.7, 71.1, 66.2, 57.1. TLC (2:1 hexanes/ethyl acetate) R_f=0.13.

To the crude benzyl alcohol (0.4 g, 1.53 mmol) in 20 mL of $CHCl_3$ at 0 °C, was added 1-methylcyclohexene (0.22 g, 2.3 mmol) followed by dropwise addition of trifluoroacetic acid (0.035 g, 1.84 mmol). The mixture was boiled for 3 h. After cooling to rt, the solution was diluted with water and extracted with CH_2Cl_2, dried, concentrated and purified by sgc using 4:1 hexanes/ethyl acetate to afford benzopyran **7** in 40% yield over two steps. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50–7.28 (m, 5H), 6.57 (s, 1H), 6.43 (s, 1H), 5.08 (s, 2H), 3.82 (s, 3H), 3.04 (dd, J=15, 6 Hz, 1H), 2.26 (d, J=15 Hz, 1H), 1.98–1.25 (m, 9H), 1.19 (s, 3H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 148.0, 147.1, 143.5, 137.5, 128.7, 127.9, 127.6, 113.9, 111.2, 103.5, 74.9, 71.1, 57.1, 38.7, 37.2, 29.2, 28.7, 25.8, 25.6, 21.9. HRMS (EI) m/z calcd for 338.1882, found 338.1887. TLC (2:1 hexanes/ethyl acetate) R_f=0.83.

1.1.5. 2-Methoxy-10a-methyl-5,6,7,8,8a,10a-hexahydro-xanthen-3-one (8). To compound **7** (0.1 g, 0.3 mmol) in 5 mL of THF at rt was added Pd/C (0.032 g, 0.03 mmol). The flask was flushed with hydrogen gas for 5 min and capped with a large balloon. After 1 h, the mixture was filtered through Celite, concentrated and purified by sgc using 4:1 hexanes/ethyl acetate. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.50 (s, 1H), 6.41 (s, 1H), 3.81 (s, 3H), 3.03 (dd, J=15, 6 Hz, 1H), 2.24 (d, J=18 Hz, 1H), 1.94–1.25 (m, 9H), 1.20 (s, 3H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 147.5, 145.0, 140.8, 112.1, 110.4, 103.8, 74.8, 56.7, 38. 7, 37.2, 29.3, 28.6, 25.8, 25.5, 21. 9; HRMS (EI) m/z calcd for 248.1412, found 232.1418. TLC (2:1 hexanes/ethyl acetate) R_f=0.56.

To the phenol (0.02 g, 0.08 mmol), in 5 mL of dioxane at rt was added DDQ (0.022 g, 0.096 mmol). After 2 h, the mixture was filtered through Celite, concentrated and purified by sgc using 4:1 hexanes/ethyl acetate to afford **8** in 95% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.23 (bs, 1H), 6.46 (s, 1H), 6.18 (s, 1H), 3.89 (s, 3H), 2.22 (dd, J=12, 3 Hz, 1H), 2.05–2.00 (m, 1H), 1.75–1.30 (m, 8H), 1.28 (s, 3H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 195.6, 156.4, 153.6, 142.0, 111.5, 106.8, 103.7, 79.8, 56.5, 52.5, 37.1, 26.9, 24.8, 24.2, 21.7. UV (CH_2Cl_2) λmax 240, 272, 335 nm; HRMS (EI) m/z calcd for 246.1256, found 232.1258. TLC (2:1 hexanes/ethyl acetate) R_f=0.43.

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References and notes

1. Kohmoto, S.; McConnell, O. J.; Wright, A.; Koehn, F.; Thompson, W.; Lui, M.; Snader, K. M. *J. Nat. Prod.* **1987**, *50*, 336.
2. El Sayed, K. A.; Bartyzel, P.; Shen, X.; Perry, T. L.; Zjawiony, J. K.; Hamann, M. T. *Tetrahedron* **2000**, *56*, 949–953.
3. Amagata, T.; Whitman, S.; Johnson, T. A.; Stessman, C. C.; Loo, C. P.; Lobkovsky, E.; Clardy, J.; Crews, P.; Holman, T. R. *J. Nat. Prod.* **2003**, *66*, 230–235. Pina, I. C.; Sanders, M. L.; Crews, P. *J. Nat. Prod.* **2003**, *66*, 2–6.
4. Hostetter, J. M.; Steadham, E. M.; Haynes, J. S.; Bailey, T. B.; Cheville, N. F. *FEMS Immunol. Med. Microbiol.* **2002**, *34*, 127–134.
5. Barrero, A. F.; Alvarez-Manzaneda, E. J.; Mar Herrador, M.; Valdivia, M. V.; Chahboun, R. *Tetrahedron Lett.* **1998**, *39*, 2425–2428.
6. Buchi, G.; Chu, P. *Tetrahedron* **1981**, *37*, 4509.
7. Harrington, P. E.; Stergiades, I. A.; Erickson, J.; Makriyannis, A.; Tius, M. A. *J. Org. Chem.* **2000**, *65*, 6576.
8. Jones, R. M.; Selenski, C.; Pettus, T. R. R. *J. Org. Chem.* **2002**, *67*, 6911–6915. Van De Water, R. W.; Pettus, T. R. R. *Tetrahedron* **2002**, *58*, 5367–5405.
9. (a) Chambers, J. D.; Crawford, J.; Williams, H.; Dufrense, C.; Scheigetz, J.; Bernstein, M. A.; Lau, C. K. *Can. J. Chem.* **1992**, *70*, 1717. (b) Chiba, K.; Sonoyama, J.; Tada, M. *J. Chem. Soc., Chem. Commun.* **1995**, 1381. (c) Diao, L.; Yang, C.; Wan, P. *J. Am. Chem. Soc.* **1995**, *117*, 5369. (d) Chiba, K.; Hirano, T.; Kitano, Y.; Tada, M. *Chem. Commun.* **1999**, 691.
10. Sayigh, A. A.; Ulrich, H.; Green, M. *J. Chem. Soc.* **1964**, 3482.
11. Krishnamurti, M.; Seshagiri, S. N. *Indian J. Chem. B.* **1976**, *14B*, 951.
12. Quideau, S.; Pouysegu, L.; Looney, M. A. *J. Org. Chem.* **1998**, *63*, 9597–9600.