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The Reaction of Tanshinones with Amines

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Abstract: The reaction of cryptotanshinone and tanshinone IIA with several biogenic amine metabolites involved in the pathogenic pathways of **HE** were investigated and eight 1,2,3,4-tetrahydrophenanthrene derivatives, **2-6** and **8-10**, were obtained. The probable mechanism on reaction was discussed.

Keywords: Cryptotanshinone, tanshinone IIA, biogenic amines.

Hepatic encephalopathy (**HE**) is a serious neuropsychiatric complication of both acute and chronic liver disease[1]. It was suggested that the abnormal high concentration of ammonia in plasma and cerebrospinal fluid and neurotransmission failure were responsible for **HE**[2,3,4,5]. In our previous work[6], cryptotanshinone, a typical diterpenoid tanshinone from the traditional Chinese medicine ***Salvia miltiorrhiza* Bunge**, has shown property of reacting with aqueous ammonia. Further animal studies showed tanshinones could decrease the ammonia concentration in plasma and alleviate the symptoms of **HE**[7]. In our attempt to explore the nature of the results, the interaction of typical tanshinones with the biogenic amine metabolites involved in the pathogenic pathways of **HE**[3,4,5], such as 2-phenyl ethylamine, tyramine, 4-aminobutyric acid and 2-amino-1-phenyl ethanol, has been investigated systematically. In this paper, we report the reaction of cryptotanshinone **1** and tanshinone IIA **7** with biogenic amine metabolites mentioned above *in vitro*.

Reactions of cryptotanshinone **1** with 2-phenyl ethylamine, tyramine and 4-aminobutyric acid gave products of **2**, **3** and **4** respectively (**Scheme 1**)[8]. According to the FAB-MS, NMR, HMQC, HMBC and elementary analysis, **2-4** can be assigned as 2-benzyl-4, 9, 9-trimethyl-4, 5, 9, 10, 11, 12-hexahydro-1, 6-dioxa-3-aza dicyclopenta [*a,c*] phenanthrene, 2-(4'-hydroxy benzyl)-4, 9, 9-trimethyl-4, 5, 9, 10, 11, 12-hexahydro-1, 6-dioxa-3-aza dicyclopenta [*a,c*] phenanthrene and 2-(2-carboxy ethyl)-4, 9, 9-trimethyl-4, 5, 9, 10, 11, 12-hexahydro-1, 6-dioxa-3-aza dicyclopenta [*a,c*] phenanthrene.

Reaction of **1** with 2-amino-1-phenyl ethanol furnished two products **5** and **6** (**Scheme 1**)[9]. According to the spectrum data, **5** can be assigned as 2-(1-hydroxy

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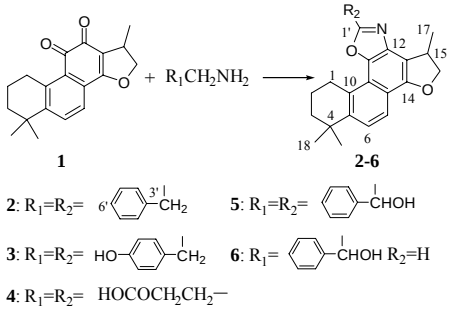
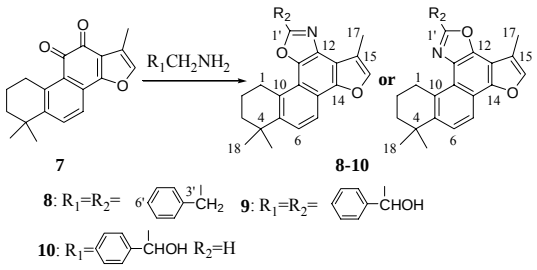
benzyl)-4, 9, 9-trimethyl-4, 5, 9, 10, 11, 12-hexahydro-1, 6-dioxa-3-aza dicyclopenta [*a,c*] phenanthrene. Regarding **6**, FAB-MS and elemental analysis indicate that its formula is $C_{20}H_{21}NO_2$. The NMR data of **6** are similar to **5** except that **6** has an additional methine group but no R_1 group (C_6H_5CHOH-) and an additional quarternary carbon signals compared with **1**, all data implying **6** is a R_1-cleaved product, 4, 9, 9-trimethyl-4, 5, 9, 10, 11, 12-hexahydro-1, 6-dioxa-3-aza dicyclopenta [*a,c*] phenanthrene.

Scheme 1



Scheme 2

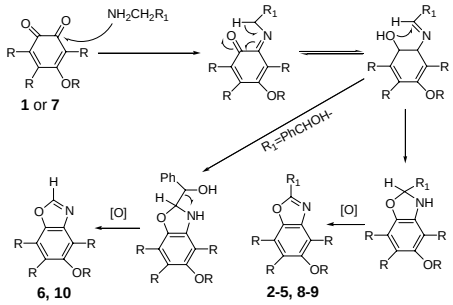


Reaction of tanshinone IIA **7** with 2-phenyl ethylamine afforded **8**. Two products, **9** and **10,** were obtained from the reaction of **7** with 2-amino-1-phenyl ethanol (**Scheme 2**)[10]. **8** and **10** can be assigned as an R_1-uncleaved and an R_1-cleaved oxazole ring derivatives respectively, according to their spectrum data. However, the exact carbon atom attached by N and O atoms in oxazole ring cannot be confirmed because the position of C-11 and C-12 cannot be assigned exactly based on HMBC and HMQC. Regarding compound **9**, no other analysis data is recorded but FAB-MS owing to limited availability.

According to the results, the possible mechanism is proposed in **Scheme 3**. The amino group of starting amine attacks the *o*-quinone moiety of tanshinone during the nucleophilic substituted reaction, and then the formed imine may run a cyclization-oxidation reaction, resulting of a molecular H_2O and two H atoms are removed. However, another product, a R_1 group (C_6H_5CHOH-) instead of a H is removed, is obtained at the same time while R_1 is C_6H_5CHOH- group. The two

pathways probably are thermodynamic competitive. Under nitrogen protection condition, **1** reacts with 2-phenyl ethylamine to give the major product **2**. That means the probable oxidant is tanshinone itself. But the reduced form of tanshinone (catechol form) is not obtain due to this intermediate is too sensitive to oxigen.

Scheme 3



The fact that tanshinones can react with those biogenic amines metabolites involved in the pathogenesis **HE** imply, to some extent, tanshinones may remove those compounds *in vivo*, which may contribute to our primary results, tanshinones can alleviate the symptoms of **HE.** Further biochemical attempts is still in progress.

Acknowledgments

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8. Data of compounds **2-4**. Compound **2**: **1** and 2-phenylethylamine were suspended in ethanol and stirred under 37°C for 10h. Colorless crystal **2** was obtained, yield 52%. $C_{27}H_{27}NO_2$, calcd: C, 81.58%; H, 6.85%; N, 3.52%; found: C, 81.56%; H, 6.89%; N, 3.49%. Mp 94.5-96^{O}C. FAB-MS *m/z* (rel. int.): 398 $[M+1]^+$ (70), 397 (100). UV λ_{max} (MeOH) 349.0, 333.5, 318.5, 255.0, 231.0nm. IR ν_{max} (KBr): 3029, 1603, 1553, 1454, 1241cm^{-1}. ^{1}HNMR (500MHz, $CDCl_3$, TMS) δ 1.35 (s, 6H), 1.53 (d, 3H, *J* 7Hz), 1.74 (m, 2H), 1.95 (m, 2H), 3.37(t, 2H, *J* 6Hz), 4.05 (m, 1H), 4.37 (dd, 1H, *J* 6, 9Hz,), 4.38 (s, 2H), 4.90 (t, 1H, *J* 9Hz), 7.25-7.28 (m, 1H), 7.32-7.35 (m, 2H), 7.40-7.43 (m, 2H), 7.47 (d, AB, 1H, *J* 9Hz), 7.83(d, AB, 1H, *J* 9Hz). ^{13}CNMR (500MHz, $CDCl_3$, TMS) δ 19.5t, 19.9q, 29.2t, 31.5q, 31.6q, 34.3s, 35.3t, 36.8d, 38.5t, 79.4t, 115.2s, 117.0s, 119.6s, 119.8d, 124.3d, 127.1d, 128.7d, 128.8d, 129.4s, 135.2s, 135.3s, 143.1s, 143.8s, 153.2s, 164.1s. Compound **3**: **1** and tyramine were suspended in ethanol and stirred under reflux for 10h. Got light yellow solid **3**, yield 17%. $C_{27}H_{27}NO_3$, calcd: C, 78.42%; H, 6.58%; N, 3.39%; found: C, 78.51%; H, 6.64%; N, 3.31%. FAB-MS *m/z* (rel. int.): 414 $[M+1]^+$ (4), 55 (100). UV λ_{max} (MeOH): 349.5, 333.5, 319.0, 255.5, 230.5nm. IR ν_{max} (KBr): 3067, 3017, 1612, 1556, 1514, 1456cm^{-1}. ^{1}HNMR (500MHz, Acetone-d_6, TMS) δ 1.35(s, 3H), 1.354 (s, 3H), 1.51 (d, 3H, *J* 7Hz), 1.75 (m, 2H),

1.94 (m, 2H), 3.39 (t, 2H, *J* 7Hz), 4.00 (m, 1H), 4.28 (s, 2H), 4.34 (dd, ABX, 1H, *J* 7, 9Hz), 4.92 (t, 1H, *J* 9Hz), 6.83 (d, AB, 2H, *J* 9Hz), 7.29 (d, AB, 2H, *J* 9Hz), 7.53 (d, AB, 1H, *J* 9Hz), 7.80 (d, AB, 1H, *J* 9Hz), 8.30 (s, 1H). ^{13}CNMR (500MHz, Acetone-d_6, TMS) δ 19.8q, 20.2t, 29.9t, 31.8q, 31.9q, 34.8t, 34.9s, 37.6d, 39.3t, 79.9t, 116.3d, 116.4s, 117.7s, 120.4s, 120.6d, 124.9d, 127.4s, 130.1s, 130.8d, 136.8s, 143.6s, 144.5s, 153.9s, 157.4s, 165.9s. Compound **4**: To a mixture of 1 and 4-aminobutyric acid in ethanol, aqueous NaOH was added and stirred under reflux for 5h. Got light brown solid **4**, yield 14%. $C_{23}H_{25}NO_4$, calcd: C, 72.80%; H, 6.64%; N, 3.69%; found: C, 72.83%; H, 6.69%; N, 3.60%. Mp 192-193°C. FAB-MS *m/z* (rel. int.): 380 $[M+1]^+$ (3), 55 (100). UV λ_{max} (MeOH) 348.0, 332.0, 318.0, 253.0, 230.0nm. IR ν_{max} (KBr): 1718, 1560, 1455, 1406cm^{-1}. ^{1}HNMR (500MHz, Acetone-d_6, TMS) δ 1.36 (s, 3H), 1.37 (s, 3H), 1.50 (d, 3H, *J* 7Hz), 1.76 (m, 2H), 1.97 (m, 2H), 3.01 (t, 2H, *J* 7Hz), 3.33 (t, 2H, *J* 7Hz), 3.44 (t, 2H, *J* 6.5Hz), 4.00 (m, 1H), 4.34 (dd, ABX, 1H, *J* 7, 9Hz), 4.92 (t, 1H, *J* 9Hz), 7.53 (d, AB, 1H, *J* 9Hz), 7.80 (d, AB, 1H, *J* 9Hz). ^{13}CNMR (500MHz, Acetone-d_6, TMS) δ 19.7q, 20.2t, 24.7t, 29.9t, 30.9t, 31.8q, 31.9q, 34.9s, 37.6d, 39.3t, 79.9t, 116.3s, 117.7s, 120.4s, 120.6d, 124.9d, 130.1s, 136.7s, 143.5s, 144.4s, 153.9s, 166.1s, 173.3s.

9. Data of compounds **5** and **6**. According to the procedure of **2**, using **1** and 2-amino-1-phenyl ethanol as starting materials. Got products **5** and **6**. Compound **5**: Light yellow solid, yield 35%. $C_{27}H_{27}NO_3$, calcd: C, 78.42%; H, 6.58%; N, 3.39%; found: C, 78.44%; H, 6.65%; N, 3.34%. FAB-MS *m/z* (rel. int.): 414 $[M+1]^+$ (50), 55 (100). UV λ_{max} (MeOH): 315.0, 335.5, 319.0, 256.0, 232.5, 208.5nm. IR ν_{max} (KBr): 3064, 3031, 1603, 1557, 1454, 1403cm^{-1}. ^{1}HNMR (500MHz, Acetone-d_6, TMS) δ 1.35 (s, 6H), 1.51 (d, 3H, *J* 7Hz), 1.76 (m, 2H), 1.95 (m, 2H), 2.87 (br, 1H), 3.41 (t, 2H, *J* 7Hz), 4.02 (m, 1H), 4.35 (dd, ABX, 1H, *J* 6.5, 9Hz), 4.92 (t, 1H, *J* 9Hz), 6.18 (s, 1H), 7.30-7.34 (m, 1H), 7.38-7.42 (m, 2H), 7.55 (d, AB, 1H, *J* 9Hz), 7.66-7.68 (m, 2H), 7.80 (d, AB, 1H, *J* 9Hz). ^{13}CNMR (500MHz, Acetone-d_6, TMS) δ 19.8q, 20.1t, 29.9t, 31.8q, 31.9q, 34.9s, 37.6d, 39.3t, 70.9d, 80.0t, 116.5s, 118.1s, 120.5s, 120.6d, 125.3d, 127.6d, 128.8d, 129.2d, 130.2s, 136.3s, 141.4s, 143.6s, 144.6s, 154.1s, 167.0s. Compound **6**: Colorless solid, yield 21%. $C_{20}H_{21}NO_2$, calcd: C, 78.15%; H, 6.89%; N, 4.56%; found: C, 78.19%; H, 6.94%; N, 4.51%. FAB-MS *m/z* (rel. int.): 308 $[M+1]^+$ (9), 55 (100). UV λ_{max} (MeOH) 348.5, 334.0, 252.0, 230.0nm. IR ν_{max} (KBr): 3069, 1602, 1505, 1457, 1399cm^{-1}. ^{1}HNMR (500MHz, Acetone-d_6, TMS) δ 1.36 (s, 3H), 1.37 (s, 3H), 1.53 (d, 3H, *J* 7Hz), 1.76 (m, 2H), 1.98 (m, 2H), 3.45 (t, 2H, *J* 7Hz), 4.04 (m, 1H), 4.36 (dd, ABX, 1H, *J* 7, 9Hz), 4.95 (t, 1H, *J* 9Hz), 7.58 (d, AB, 1H, *J* 9Hz), 7.83 (d, AB, 1H, *J* 9Hz), 8.52 (s, 1H). ^{13}CNMR (500MHz, Acetone-d_6, TMS) δ 19.7q, 20.1t, 29.9t, 31.8q, 31.9q, 34.9s, 37.6d, 39.3t, 80.0t, 116.5s, 118.4s, 120.4s, 120.6d, 125.4d, 130.3s, 135.7s, 143.0s, 144.7s, 153.5d, 154.2s.

10. Data of compounds **8-10**.
Mixture of **7** and amine in ethanol was stirred under reflux for 25h.
Using 2-phenyl ethylamine as starting material, got colorless solid **8**, yield 58%. $C_{27}H_{25}NO_2$, calcd: C, 82.00%; H, 6.37%; N, 3.54%; found: C, 81.8%; H, 6.40%; N, 3.51%. Mp 132-133^{O}C. FAB-MS *m/z* (rel. int.): 396 $[M+1]^+$ (100%). UV λ_{max} (MeOH): 343.0, 327.0, 312.5, 280.0, 264.5, 257.0nm. IR ν_{max} (KBr): 3031, 1603, 1551, 1454, 1382, 1238cm^{-1}. ^{1}HNMR (500MHz, Acetone-d_6, TMS) δ 1.38 (s, 6H), 1.76 (m, 2H), 1.97 (m, 2H), 2.54 (d, 3H, *J* 1.5Hz), 3.44 (t, brd, 2H, *J* 6.5Hz), 4.46 (s, 2H), 7.26-7.30 (m, 1H), 7.35-7.39 (m, 2H), 7.48-7.51 (m, 2H), 7.67 (d, AB, 1H, *J* 9Hz), 7.77 (q, 1H, *J* 1.5Hz), 8.12 (dt, 1H, *J* 9Hz). ^{13}CNMR (500MHz, Acetone-d_6, TMS) δ 9.3q, 20.2t, 30.0t, 32.0q, 35.0s, 35.6t, 39.2t, 117.1s, 117.2s, 118.4s, 118.6s, 119.0d, 126.1d, 127.8d, 129.5d, 129.8d, 130.8s, 133.9s, 136.8s, 142.6d, 144.1s, 145.2s, 150.0s, 165.2s.
Using 2-amino-1-phenyl ethanol as starting material, got products **9** and **10**. Compound **9**: FAB-MS *m/z* (rel. int.): 412 [M+1]+ (12), 154 (100). Owing to limited availability, no other spectrum was recorded. Compound **10**: Colorless solid, yield 40%. $C_{20}H_{19}NO_2$, calcd: C, 78.66%; H, 6.27%; N, 4.59%; found: C, 78.65%; H, 6.29%; N, 4.56%. Mp134-138 ^{O}C. FAB-MS *m/z* (rel. int.): 306 $[M+1]^+$ (60), 55 (100). UV λ_{max} (MeOH): 342.0, 326.5, 261.5, 255.0nm. IR ν_{max} (KBr): 3066, 1629, 1505, 1452, 1382cm^{-1}. ^{1}HNMR (500MHz, Acetone-d_6, TMS) δ 1.41 (s, 6H), 1.81 (m, 2H), 2.02 (m, 2H), 2.56 (d, 3H, *J* 1.5Hz), 3.54 (t, brd, 2H, *J* 6.5Hz), 7.74 (d, 1H, *J* 9Hz), 7.81 (q, 1H, *J* 1.5Hz), 8.16 (dt, 1H, *J* 1, 9Hz), 8.64 (s, 1H). ^{13}CNMR (500MHz, Acetone-d_6, TMS) δ 9.3q, 20.1t, 30.1t, 32.0q, 35.1s, 39.2t, 117.2s, 117.3s, 118.5s, 119.0d, 119.1s, 126.5d, 131.2s, 132.8s, 142.8d, 144.3s, 145.0s, 150.2s, 153.5d.

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