PREPARATION AND ANTILEUKEMIC ACTIVITY OF CONGENERS OF TROPOLOISOQUINOLINE ALKALOIDS FROM *ABUTA CONCOLOR*

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Abstract - Antileukemic tropoloisoquinoline alkaloids, pareirubine A (**1**) and grandirubrine (**2**), at first have been isolated from *Abuta concolor* (Menispermaceae). Methylation of **1** and **2**, existing in solution as a mixture of tautomers gave the corresponding four methyl derivatives (**3** - **6**). Thioimerubrine (**7**) and thioisoimerubrine (**8**) were prepared by the nucleophilic substitutions of the methoxyl groups at C-11 and -10, respectively. Acetylation of **1** and **2** produced the corresponding mono-acetyl tautomers (**9** and **10**). Antileukemic activity of these derived tropoloisoquinoline alkaloids is also reported.

During the course of our investigation in search of new antitumor substances from South American plants,[1] we found antineoplastic activity of methanolic extract from ABUTA (Brazilian plant name), as the original plants of which, the tropical American genera *Abuta, Chondodendron,* and *Cissampelos et al.* (Menispermaceae) are known. These plants also are well known as a part of Amazonian curare ingredients.[2]

The ABUTA plant obtained at Belém in Brazil by us was identified as *Abuta concolor* SOERR. When the methanolic extract was partitioned between methylene chloride and water, the cytotoxic activity against P388 leukemia cells passed over into the methylene chloride fraction, which was further separated by chromatographic purification with medium-pressure liquid chromatography (mplc) and hplc using reversed-phase ODS column with the guidance of bio-assay against P388 cells. The bio-active principles were

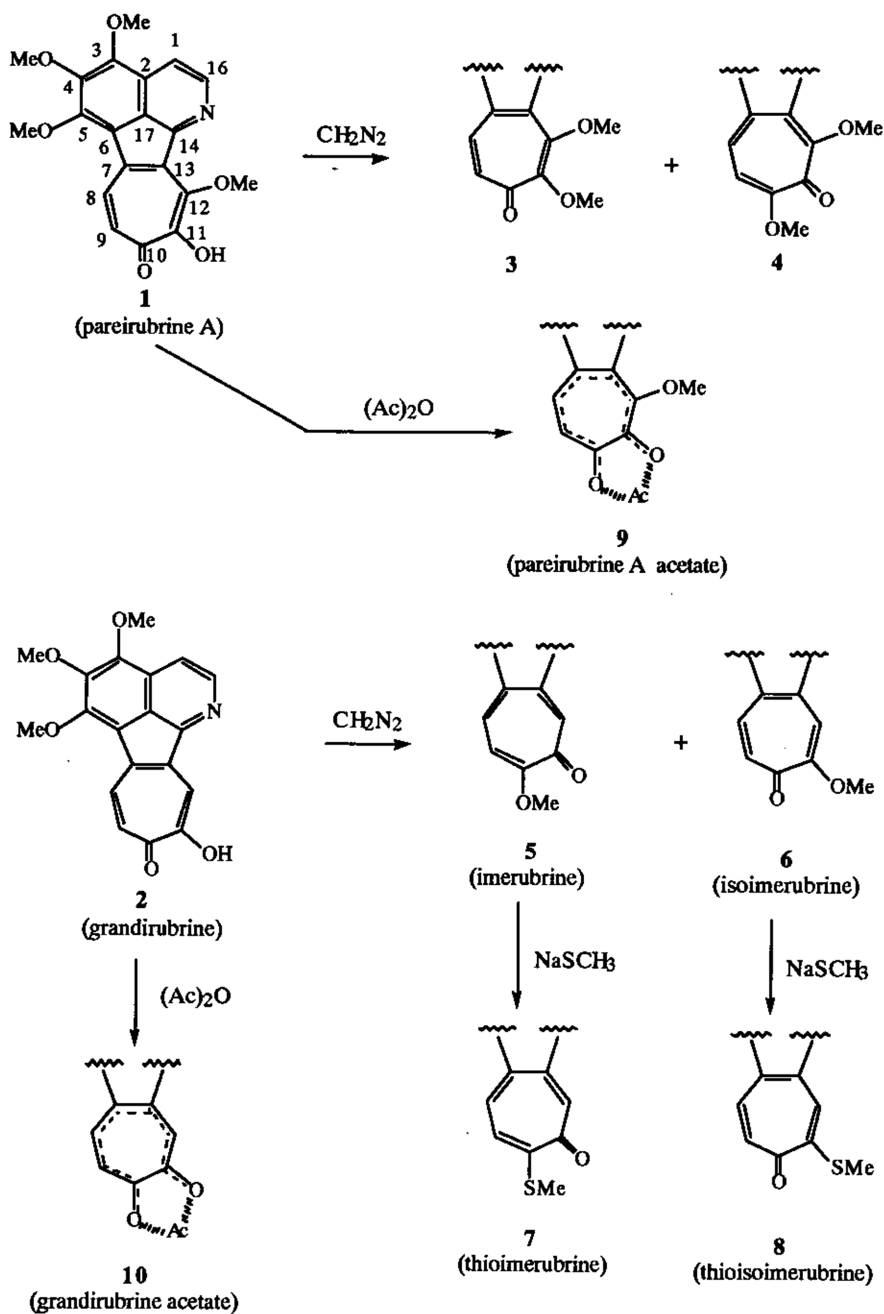

Figure 1 Various tropoloisoquinoline alkaloids derived from pareirubrine A and grandirubrine

at first confirmed to be tropoloisoquinoline alkaloids, pareirubrine A (**1**) and grandirubrine (**2**) by direct comparison with their authentic samples.[3, 4] Following the identification of antineoplastic principles from *A. concolor,* we attempted to prepare several derivatives of **1** and **2** in order to elucidate the structure - activity relationships, because the importance has been reported in colchicine and its congeners containing tropolone skeleton in their partial structures.[5, 6]

Reaction of pareirubrine A (**1**) with excess diazomethane in methanol afforded a mixture of two isomeric methyl ethers, (**3**) and (**4**) (2:1). The major (**3**) and minor (**4**) ethers were determined to be 11-methylpareirubrine A and 10-methylpareirubrine A respectively as shown in Figure 1, because the ^{1}H-nmr chemical shifts at the positions 8 and 9 were similar to those of isoimerubrine (**6**, δ 8.25 and 7.38) in **3** (δ 8.32 and 7.38) and imerubrine (**5**, δ 8.03 and 6.85) in **4** (δ 8.12 and 6.93). Acetylation of **1** with acetic anhydride and pyridine gave the mono-acetates (**9**), which were a mixture of the tautomers consisting of 10- and 11-acetylpareirubrines A as reported in colchicein acetate.[7]

The structure of grandirubrine (**2**) was also assigned and derived by conversion of the alkaloid with excess diazomethane to a mixture of imerubrine (**5**) and isoimerubrine (**6**) (1:1), which were confirmed by comparing their physical and spectral data with those of the literatures.[8, 9] Grandirubrine acetate (**10**) was prepared by acetylation of **2** with acetic anhydride and pyridine in the usual way. Also, thioimerubrine (**7**) and thioisoimerubrine (**8**) directly were prepared by treating **5** and **6** with sodium methanethiolate according to A. Brossi's method.[10] The positions of thiomethoxyl groups were confirmed by observing the nOe between methyl-S (δ 2.47, s) and 9-positional (δ 7.10, d, J=10 Hz) protons in **7**, and between methyl-S (δ 2.66, s) and 12-positional (δ 8.21, s) protons in **8**.

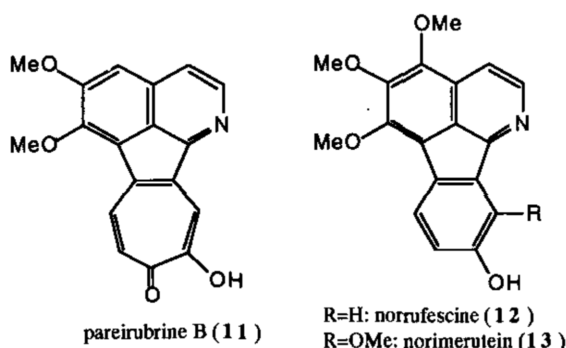

Figure 2 Tropoloisoquinoline and azafluoranthene alkaloids from other Amazonian Abuta plant, *Cissampelos pareira*

Table 1 Cytotoxic activity against P388 cells

	IC50 (μg/ml)		IC50 (μg/ml)
1	0.33	**8**	0.45
2	0.18	**9**	0.49
3	0.33	**10**	0.16
4	1.40	**11**	0.17
5	1.20	**12**	5.80
6	0.65	**13**	3.60
7	0.45		

The compounds (**1** - **10**) and their related alkaloids (**11** - **13**) which were isolated from *Cissampelos pareira* (Menispermaceae, Brazilian name ABUTA)[4, 11] were subjected to the cytotoxic bio-assay against P388 leukemia cells. As can be seen from Table 1, it was proved that tropoloisoquinoline alkaloids containing 10-carbonyltropolone core showed stronger cytotoxicity than 11-carbonyltroponoid alkaloids and also, tropoloisoquinoline alkaloids exhibited more significant activity than azafluoranthene alkaloids.

EXPERIMENTAL

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Infrared (ir) spectra were taken on a Perkin Elmer 1710 or a JASCO A-302 spectrophotometer. ^{1}H and ^{13}C-nmr spectra were recorded on a Bruker AM-400 spectrometer. Chemical shifts were expressed in ppm with tetramethylsilane as an internal standard. The mass spectra (ms) were taken with Hitachi M-80 and VG AutoSpec spectrometer. The ultraviolet (uv) and visible absorption spectra were recorded on Hitachi 557 spectrophotometer. Mplc was performed with a CIG column system (22 mm i.d. x 300 mm, Kusano Scientific Co., Tokyo) packed with 10 μm silica gel or 20 μm ODS. Hplc was performed with an Inertsil PREP-ODS column (20 mm i.d. x 250 mm, Gasukuro Kogyo Inc.) packed with 10 μm ODS. Tlc was conducted on precoated Kieselgel 60 F_{254} (Art. 5715, Merck) and each spot was detected by heating after spraying with 10% H_2SO_4.

Materials. The stems of *Abuta concolor* SOERR. were purchased at Belém, Brazil. The botanical identification was made by Dra. Irenice Alves Rodrigues (Centro de Pesq. Agroflorestal da Amazônia Oriental of EMBRAPA at Belém, Brazil). A voucher specimen has been deposited in the herbarium of Tokyo College of Pharmacy.

Extraction and isolation The stems (580 g) of *A. concolor* were crushed and extracted with boiling hot MeOH - CH_2Cl_2 (1:1) (2 l) for 5 h to give the extract (40 g) which was partitioned between CH_2Cl_2 (800 ml) and H_2O (500 ml). The CH_2Cl_2 soluble fraction (3.1 g) was subjected to CIG column packed with ODS using MeOH to give two fractions. Second fraction (225 mg) was further subjected to ODS hplc using MeOH - MeCN - potassium phosphate buffer (pH 3.5) (2:2:1) to furnish pareirubrine A (**1**, 10 mg) and grandirubrine (**2**, 28 mg), which were identified by direct comparison with their authentic samples.[4]

Methylation of pareirubrine A (1) A solution of pareirubrine A (15 mg) in MeOH (5 ml) was treated with excess diazomethane at room temperature for 6 h. The concentrated reaction mixture was subjected to

silica gel CIG column using CH_2Cl_2 - MeOH (37:1) to give 11-methylpareirubrine A (**3**, 7.8 mg) and 10-methylpareirubrine A (**4**, 4.2 mg), respectively. **3**: orange-red needles, mp 139 - 141°C; eims *m/z* (%): 381 (M^+, 67), 353 (34), 338 (100), 308 (74), 237 (35); ir ($CHCl_3$) cm^{-1}: 1610, 1420, 1330, 1120; uv (EtOH) nm (ε): 220 (25700), 254 (17800), 324 (11900), 360 (20300), 406 (6200, sh), 428 (7500), 480 (2700, sh); ^{1}H-nmr ($CDCl_3$, δ): 4.01, 4.05, 4.11, 4.20, 4.21 (3H, s, -OMe,respectively), 7.37 (1H, d, J=12.2 Hz, H-9), 7.78 (1H, d, J=5.7 Hz, H-1), 8.32 (1H, d, J=12.2 Hz, H-8), 8.89 (1H, d, J=5.7 Hz, H-16). **4**: yellow-red needles, mp 78 - 80°C; eims *m/z* (%): 381(M^+, 95), 364 (100), 338 (39), 308 (44), 280 (33); ir ($CHCl_3$) cm^{-1}: 1610, 1580, 1480, 1460, 1090; uv (EtOH) nm (ε): 218 (20600), 246 (18400), 350 (6800), 460 (2400, sh); ^{1}H-nmr ($CDCl_3$, δ): 4.01, 4.06, 4.13, 4.15, 4.19 (3H, s, -OMe,respectively), 6.93 (1H, d, J=10.2 Hz, H-9), 7.72 (1H, d, J=5.8 Hz, H-1), 8.12 (1H, d, J=10.2 Hz, H-8), 8.82 (1H, d, J=5.8 Hz, H-16).

Acetylation of 1 A solution of pareirubrine A (**1**, 6 mg) in pyridine (2 ml) was stirred with acetic anhydride (1 ml) at room temperature for 24 h. Then, the reaction mixture was added to ice-cold water and was stirred. After that, pareirubrine A acetate (**9**, 7.2 mg) was extracted with CH_2Cl_2. **9**: yellow needles, mp 118 - 120°C; eims *m/z* (%): 409 (M^+, 26), 367 (94), 352 (100), 324 (88), 308 (48), 296 (61), 266 (66), 238 (60); ir ($CHCl_3$) cm^{-1}: 1760, 1600, 1580, 1420, 1300; uv (EtOH) nm (ε): 218 (25800), 274 (22100), 300 (14700), 368 (8400), 398 (6200), 450 (8100); ^{1}H-nmr ($CDCl_3$, δ): 2.41 (3H, s, COMe), 4.05, 4.16, 4.16, 4.18, (3H, s, -OMe,respectively), 7.36 (1H, d, J=9.9 Hz, H-9), 7.74 (1H, d, J=5.8 Hz, H-1), 8.08 (1H, d, J=9.9 Hz, H-8), 8.83 (1H, d, J=5.8 Hz, H-16).

Methylation of grandirubrine (2) A solution of grandirubrine (12 mg) in MeOH (5 ml) was reacted with excess diazomethane at room temperature for 6 h. The concentrated reaction mixture was subjected to ODS hplc using H_2O - MeOH (1:3) to give isoimerubrine (**6**, 3.3 mg) and imerubrine (**5**, 3.2 mg), which were identified by comparing their physical and spectral data with those of the literatures.[8, 9] **5**: yellow-red needles, mp 183 - 185°C. **6**: orange-red needles, mp 183 - 185°C.

Acetylation of 2 A solution of grandirubrine (**2**, 5 mg) in pyridine (2 ml) was stirred with acetic anhydride (1 ml) at room temperature for 12 h. Then, the reaction mixture was added to ice-cold water and was stirred. After that, grandirubine acetate (**10**, 3.8 mg) was extracted with CH_2Cl_2 and was purified with ODS hplc using the eluent MeCN - H_2O (11:9). **10**: yellow needles, mp 169 - 1171°C; eims *m/z* (%): 379 (M^+, 6), 337 (100), 322 (22), 294 (36), 251 (25), 208 (24), 180 (17), 152 (15); ir ($CHCl_3$) cm^{-1}: 1770, 1610, 1590, 1460, 1410, 1370, 1150, 1080, 1020, 930; uv (EtOH) nm (ε): 220 (24300), 272 (21700), 308 (11700, sh),

326 (11100, sh), 350 (11900), 424 (9900); ^{1}H-nmr ($CDCl_3$, δ): 2.40 (3H, s, COMe), 4.05, 4.18, 4.19, (3H, s, -OMe,respectively), 7.34 (1H, d, J=10.4 Hz, H-9), 7.78 (1H, d, J=5.8 Hz, H-1), 8.07 (1H, d, J=10.4 Hz, H-8), 8.29 (1H, s, H-12), 8.70 (1H, d, J=5.8 Hz, H-16).

Preparation of thioimerubrine (7) Sodium methanethiolate (20 mg) was added to a solution of imerubrine (**5**, 5 mg) in water (2 ml). The reaction mixture was stirred at room temperature for 48 h, diluted with 2% acetic acid solution (5 ml) and then extracted with CH_2Cl_2. The extract was subjected to silica gel CIG column chromatography using the eluent CH_2Cl_2 - MeOH (30:1) to give **7** (1.5 mg). **7**: yellow-red needles, mp 69 - 71°C; fabms *m/z* (%): 368 (M^++1, 93), 354 (27), 340 (20), 310 (19), 277 (15), 182 (100); ir ($CHCl_3$) cm^{-1}: 1590, 1480, 1460, 1410, 1020, 930; uv (EtOH) nm (ε): 212 (24500), 246 (21200), 270 (15700, sh), 300 (11000, sh), 370 (11000), 400 (7100, sh), 424 (8400); ^{1}H-nmr ($CDCl_3$, δ): 2.47 (3H, s, SMe), 4.07, 4.15, 4.17, (3H, s, -OMe,respectively), 7.10 (1H, d, J=10.0 Hz, H-9), 7.76 (1H, d, J=5.8 Hz, H-1), 8.07 (1H, d, J=10.0 Hz, H-8), 8.13 (1H, s, H-12), 8.69 (1H, d, J=5.8 Hz, H-16).

Preparation of thioisoimerubrine (8) Sodium methanethiolate (20 mg) was added to a solution of isoimerubrine (**6**, 5 mg) in water (2 ml). The reaction mixture was stirred at room temperature for 48 h. Work-up of the reaction mixture similar to that noted for **7** gave **8** (1.5 mg). **8**: orange-red needles, mp 52 - 54°C; fabms *m/z* (%): 368 (M^++1, 82), 354 (50), 340 (68), 277 (100); ir ($CHCl_3$) cm^{-1}: 1610, 1590 1480, 1460, 1420, 1130; uv (EtOH) nm (ε): 216 (23500), 244 (17400, sh), 264 (14700, sh), 344 (10100, sh), 436 (3800), 520 (2800, sh); ^{1}H-nmr ($CDCl_3$, δ): 2.66 (3H, s, SMe), 4.03, 4.19, 4.22, (3H, s, -OMe,respectively), 7.22 (1H, d, J=12.0 Hz, H-9), 7.79 (1H, d, J=5.7 Hz, H-1), 8.21 (1H, s, H-12), 8.31 (1H, d, J=12.0 Hz, H-8), 8.76 (1H, d, J=5.7 Hz, H-16).

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