A CHEMISTRY OF BISBENZYLISOQUINOLINE ALKALOID: ISOMERIZATION OF BERBAMINE TO PENDULINE IN METHANOL BY RADICAL REACTION

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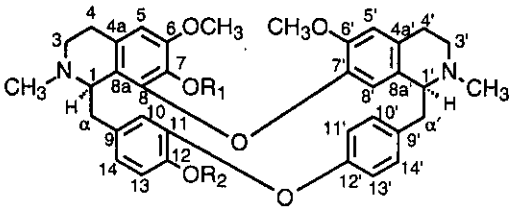
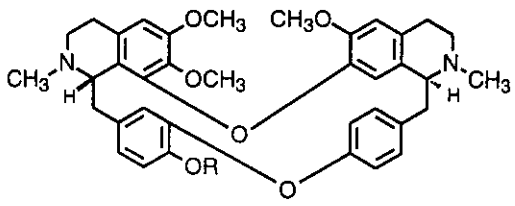
Abstract- Berbamine (**1**), a bisbenzylisoquinoline alkaloid, when treated with methanol, was isomerized to penduline (**2**), the C-1 epimer of **1**. This isomerization was proved to proceed *via* a radical reaction by a free radical generated from methanol and molecular oxygen.

In a study of determination of bisbenzylisoquinoline alkaloids, we prepared the methanol solution of berbamine (**1**) as a standard solution and observed that **1** underwent an isomerization in methanol. In this paper we deal with this subject in details.

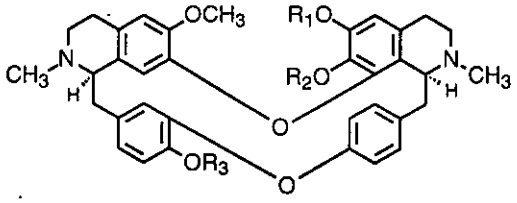
When **1** in methanol was heated for 24 hours at 40°C or allowed to stand for 2 days at room temperature, a new peak (peak II) beside **1** (peak I) appeared in *ca* 2.5 : 1 ratio for peaks I and II in the hplc chromatogram (Figure 1a). When the solution was further heated at 40°C for a long time, the ratio of the two peaks did not change though several other small peaks gradually appeared. The product (peak II) was readily isolated from the methanol solution by silica gel column chromatography. Although the high resolution mass and ^{1}H- and ^{13}C-nmr spectra indicated that this is the stereoisomer of **1**, the ^{1}H-nmr spectrum data (Table 1) measured at 24°C were not identical with any reported one of known stereoisomers of **1**. However, we found that the data obtained at 40° and 60°C were well consistent with those of penduline



1: $R_1 = CH_3$, $R_2 = H$
4: $R_1 = R_2 = CH_3$
5: $R_1 = R_2 = H$



2: R = H
3: $R = CH_3$



6: R_1 - R_2 = - CH_2 -, $R_3 = CH_3$
7: R_1 - R_2 = - CH_2 -, $R_3 = H$
8: $R_1 = R_3 = CH_3$, $R_2 = H$

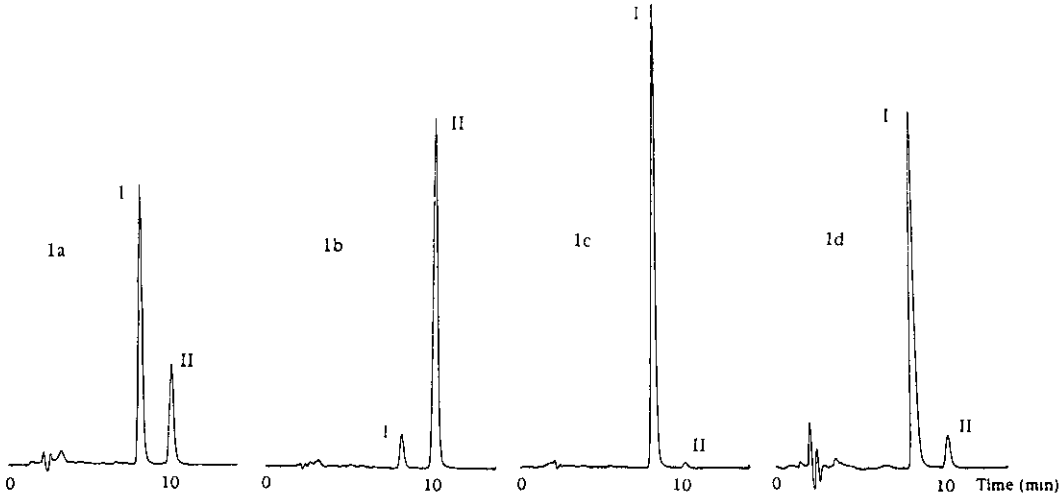


Figure 1 Hplc chromatograms (I : berbamine, II : penduline)

hplc conditions column; LiChroCART LiChrospher 100 RP-18(e) 4 x 250 mm
solvent; CH_3OH-CH_3CN-H_2O(pH 6.7) containing 0.1 M H_3PO_4 (1:2:2: v/v)
flow rate; 1.0 ml/min, detection; uv 282 nm

(**2**)[1] reported by Hussain *et al.*[1a] and Khan *et al.*[1b], respectively (Table 2). Methylation of this product with diazomethane gave tetrandrine (**3**), whose identity was finally confirmed by direct comparison with the authentic sample of **3** (ir and ^{1}H-nmr).[2]

Therefore, it was proved that treatment of **1** with methanol caused epimerization at C-1 position to produce **2**. Interestingly, treatment of **2** with methanol under a similar condition also caused the same epimerization, though in lesser extent as shown by hplc chromatogram (Figure 1b). Thus, this reaction was found to be reversible.

Table 1 ^{1}H(500 MHz)- and ^{13}C(125 MHz)-Nmr assignments of **2**

(at 24°C, in $CDCl_3$, δ in ppm)

location	^{1}H	^{13}C	location	^{1}H	^{13}C
C-1	3.96 m	60.98	C-1'	3.93 dd(10.8, 6.1)	63.44
N-CH_3	2.32 s	41.56	N'-CH_3	2.62 s	42.49
C-3	2.94 m	43.42	C-3'	2.92 m	45.10
	3.58 m			3.46 m	
C-4	2.45 m	21.61	C-4'	2.76 m	25.29
	2.92 m			3.00 m	
C-4a	6.29 s	127.52	C-4a'		127.64
C-5		105.83	C-5'	6.46 s	112.44
C-6		151.38	C-6'		148.55
C-7		137.84	C-7'		143.55
C-8		148.22	C-8'	6.08 s	120.18
C-8a		122.83	C-8a'		127.79
C-α	2.53 d(14.3)	41.69	C-α'	2.77 dd(12.5,10.8)	38.02
	2.72 m			3.28 dd(12.5,6.1)	
C-9		132.92	C-9'		135.06
C-10	6.42 d(1.5)	115.14	C-10'	6.06 dd(8.1,1.5)	132.81
C-11		143.73	C-11'	6.58 dd(8.1,2.4)	122.45
C-12		147.78	C-12'		153.15
C-13	6.73 d(8.1)	116.51	C-13'	7.02 dd(8.1,2.4)	121.77
C-14	6.76 dd(8.1,1.5)	123.14	C-14'	7.29 dd(8.1,1.5)	130.15
6-OCH_3	3.74 s	55.79	6'-OCH_3	3.16 s	55.31
7-OCH_3	3.20 s	60.20			

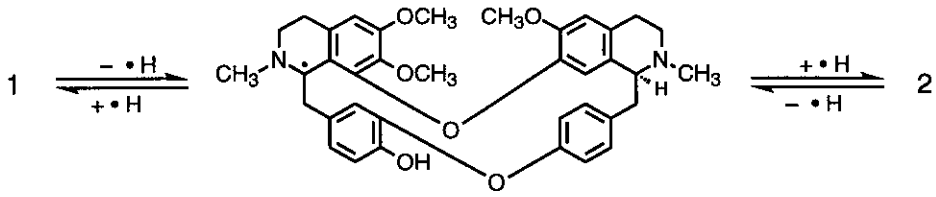
Ethanol also caused the same epimerization but in much lesser extent (Figure 1c), but in aprotic solvents such as acetone, benzene, ethyl acetate, tetrahydrofuran, acetonitrile, dimethylformamide, and dimethylsulfoxide this reaction was not observed at all. This fact indicated that a protonic solvent is required for the reaction.

The scope for this reaction seemed to be limited to the cases of **1** and **2**, since other bisbenzylisoquinoline alkaloids, tetrandrine (**3**), isotetrandrine (**4**), obamegine (**5**), cepharanthine (**6**), cepharanoline (**7**), and homoaromoline (**8**), did not produce the corresponding C-1 epimer when they were treated with methanol under a similar condition.

Formation of **2** from **1** in methanol was depressed by addition of either ascorbic acid or α-tocopherol. Furthermore, oxygen-free methanol prepared by bubbling nitrogen completely depressed this epimerization. While, methanol saturated with oxygen accelerated the reaction rates in *ca* 4 times, revealing that oxygen participates to the reaction. Thus, the epimerization at C-1 should proceed *via* a combined reaction of oxidation (hydrogen abstraction) and reduction (hydrogen addition). Hydrogen peroxide and formaldehyde were eliminated as possible reactants since addition of them to a methanol solution of **1** did not give **2** at all, respectively, instead only produced several other unidentified polar products.

Table 2 ^{1}H-Nmr chemical shifts of **2** measured at 40° and 60°C (in $CDCl_3$, δ in ppm)

40°C	Hussain *et al.*[1a]	60°C	Khan *et al.*[1b]
2.33	2.32	2.32	2.34
2.62	2.63	2.61	2.63
3.19	3.19	3.19	3.19
3.24	3.23	3.29	3.29
3.74	3.75	3.73	3.75
6.01	6.03	6.00	6.01
6.16	6.13	6.23	6.20
6.29	6.30	6.29	6.30
6.46	6.45	6.48	6.48
6.48	6.48	6.50	6.49
6.65	6.64	6.70	6.70
6.31	7.32	7.31	7.34
6.78	6.79	6.81	6.82
6.81	6.79	6.81	6.82
7.06	7.05	7.08	7.09
7.31	7.32	7.31	7.34



Scheme 1

Besides ascorbic acid or α-tocopherol, 1,1-diphenyl-2-picrylhydrazyl (DPPH) of a typical radical scavenger stopped the reaction completely, while 2,2'-azobisisobutyronitrile (AIBN) of a radical initiator when added to the oxygen-free methanol solution of **1** caused the epimerization (Figure 1d). However, treatment of **1** with AIBN in acetonitrile at 60°C or tributyltin hydride (Bu_3SnH) - AIBN in oxygen-free dioxane at 80°C did not cause the reaction at all. On the basis of the accumulated results described above, the isomerization between **1** and **2** can be rationalized in terms of a radical reaction induced by a free radical such as $\cdot CH_2OH$ or $CH_3O\cdot$ generated from methanol and molecular oxygen. The epimerization at C-1 would probably proceed through direct abstraction of C-1 hydrogen by the radical species and addition of hydrogen radical to the radical intermediate. (Scheme 1) The abstraction of C-1 hydrogen should be facilitated by participation of the neighboring nitrogen and aromatic ring.

It is very interesting that **1** and **2** of bisbenzylisoquinoline alkaloids having such relative large and complex structures caused the isomerization in a selective manner under such mild conditions.

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EXPERIMENTAL

Conversion of 1 to 2

A solution of **1** (996 mg) in MeOH (100 ml) was permitted to stand for 24 h at 40°C. The solution was evaporated *in vaccuo* below 40°C. The residue was subjected to silica gel column chromatography using 5% acetone-$CHCl_3$ and 20% acetone-$CHCl_3$ as eluents to afford **2** (245 mg, 25%) and **1** (677 mg, 68%), respectively.

Penduline(2) (peak II)

mp 148-149°C colorless needles (acetone). $[\alpha]_D^{28°}$ +265°(*c* 0.52, $CHCl_3$). Uv(MeOH) λ_{max} nm(log ε): 282(3.80). Ir(KBr) cm^{-1}: 3368, 2934, 1607, 1510, 1450, 1415, 1357, 1272, 1226, 1166, 1116, 1069, 1021. LR-ms m/z(%): 608(M^+ 100), 607(58), 396(18), 395(54), 381(29), 379(13), 364(5), 198(5), 192(13), 174(8). HR-ms: 608.2913 ($C_{37}H_{40}N_2O_6$ requires 608.2886). Cd(dioxane) Δε(nm): +10.0 (289), -14.3 (248), +21.8(237). ^1H- and ^{13}C-Nmr: Table 1.

Methylation of 2

To a solution of **2** (50 mg) in MeOH (5 ml) was added CH_2N_2-Et_2O solution under ice-cooling, and then the solution was stirred for 24 h. Work-up of the product as usual manner afforded **3** (quant.), as colorless needles (mp 150-151°C) from acetone, which was found to be identical with authetic sample by comparison of tlc and hplc behaviors, and ir and ^1H-nmr spectra.

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