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Studies on the Alkaloids of Menispermaceous Plants. CCLXXV.[1] Syntheses of [methylenedioxy-^{14}C]Cepharanthine and a Related Compound

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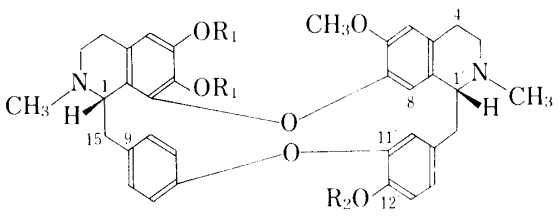
[methylenedioxy-^{14}C]Cepharanthine was synthesized *via* the selective demethylenation of cepharanthine and reconstruction of cepharanthine by methylenation of the demethylenated product with [^{14}C]methylene iodide. A derivative of cepharanthine which has an ethylenedioxy group in the place of the methylenedioxy group was prepared for examination of biological activity.

Keywords——cepharanthine; demethylenated cepharanthine; selective demethylenation; methylenation with CsF; [methylenedioxy-^{14}C]cepharanthine; [^{14}C]methylene iodide; ethylenedioxy derivative of demethylenated cepharanthine

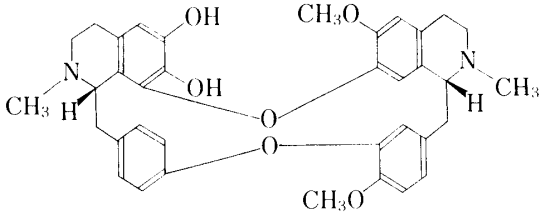
It is well known that cepharanthine (**1**), one of the bisbenzylisoquinoline alkaloids, has significant biological activities[2] and is in medicinal use.[3] In order to the study biological behavior of cepharanthine (**1**) in more detail, preparation of whole-body autoradiograms of experimental animals after drug administration is desirable. However, generally speaking, it has been regarded as difficult to insert a radioactive atom into an appropriate position of a natural product having a complex structure, because reconstruction of the original molecule having a radioactive atom from selectively degraded product is required. In the present case, [12′-*O*-methyl-^{14}C]cepharanthine (**1a**) could be prepared from naturally occurring cepharanoline[4] [12′-*O*-demethylcepharanthine] (**2**), so the former (**1a**) might be available for this purpose. However, randomization of radioactivity might occur since cepharanthine (**1**) is transformed mainly into cepharanoline (**2**) *in vivo*.[5] In this report, we describe the synthesis of [methylenedioxy-^{14}C]cepharanthine (**1b**) *via* selective demethylenation of naturally occurring cepharanthine (**1**) followed by methylenation with [^{14}C]methylene iodide (**3**) in the presence of anhydrous cesium fluoride. A related derivative (**4**) having an ethylenedioxy group instead of the methylenedioxy group of cepharanthine (**1**) was also synthesized by treatment of the demethylenated product (**5**) with ethylene dibromide in the presence of the same reagent.

In 1967, Tomita *et al.*[6] succeeded in preparing demethylenated cepharanthine (**5**) by treatment of cepharanthine (**1**) with phloroglucinol in 50% sulfuric acid in 28.1% yield. Recently, Teitel *et al.*[7] reported selective demethylenation of compounds having methylenedioxy and methoxy groups by treatment with boron trichloride. On the other hand, Clark *et al.*[8] recently reported methylenation of catechol derivatives by treatment with methylene halide in the presence of anhydrous cesium fluoride or anhydrous potassium fluoride in *N*,*N*-dimethylformamide (DMF). We, therefore, attempted to apply these reactions to cepharanthine (**1**).

Treatment of cepharanthine (**1**) with boron trichloride in 8 mol ratio at −40— −35°C according to the reported procedure[7] gave the desired demethylenated derivative (**5**) as a labile amorphous mass[6] in 73.7% yield. Although all attempts at derivation of this product (**5**) to a crystalline salt failed, its structure was established by the fact that, in the proton nuclear magnetic resonance (^{1}H-NMR) spectrum, it shows two methoxy signals at δ 3.57 and 3.87 as singlets but no signal attributable to a methylenedioxy group.



1 : $2R_1 = CH_2$, $R_2 = CH_3$
1a : $2R_1 = CH_2$, $R_2 = {}^{14}CH_3$
1b : $2R_1 = {}^{14}CH_2$, $R_2 = CH_3$
2 : $2R_1 = CH_2$, $R_2 = H$



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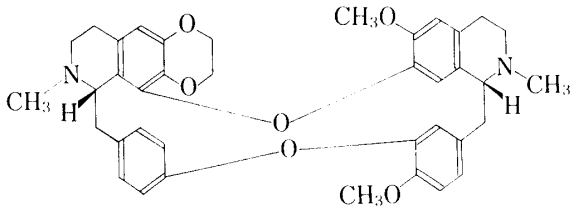

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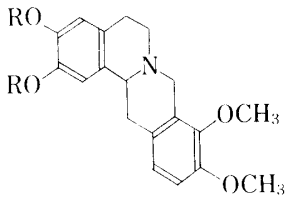

6 : R = H
7 : 2R = CH_2

Chart 1

Before methylenation of demethylenated cepharanthine (**5**), we examined the methylenation of demethylenated (±)-canadine[7b] (**6**) in order to choose suitable reaction conditions. According to the reported procedure,[8] treatment of the demethylenated compound (**6**) with anhydrous cesium fluoride and methylene bromide in DMF gave (±)-canadine (**7**) in 88.1% yield, while similar treatment using anhydrous potassium fluoride instead of anhydrous cesium fluoride as a reagent was unsatisfactory.

We examined the reaction conditions for the methylenation of demethylenated cepharanthine (**5**) in detail. First, demethylenated cepharanthine (**5**) was treated with 1.1 equivalents of methylene bromide or 1.0 equivalent of methylene iodide in the presence of an excess amount of anhydrous cesium fluoride to give cepharanthine (**1**) in 27.0 or 24.0% yield, respectively. Only a minute quantity of cepharanthine (**1**) could be obtained when anhydrous potassium fluoride was used instead of anhydrous cesium fluoride. The cepharanthine produced is identical with a sample of naturally occurring cepharanthine even on optical rotation. These results indicate that the difference of reagent species between these alkyl halides does not affect the yield of the product when anhydrous cesium fluoride is used as the fluoride salt in this reaction. Since [^{14}C]methylene iodide (**3**) is easier to handle than [^{14}C]methylene bromide in the preparation of the labelled compound because of a higher boiling point, we decided to

Table I. Methylenation Yield of Demethylenated Cepharanthine (**5**)

Species of halogen	Material		Yield[a] (%)	
	CH_2X_2 (eq)	**5** (eq)	Based on **5**	Based on CH_2X_2
X=Br	1.1	1.0	27.0(55.3)[b]	—
X=I	1.0	1.0	24.0(44.4)	24.0(44.4)
	1.0	3.0	—	61.3(quant.)
	1.0	6.0	—	51.3(quant.)

a) The material shows one spot on TLC. The crude material could be used in the following step without recrystallization.
b) ( ): Yield before recrystallization.

use the former (**3**) in our experiment. We carried out the reaction using an excess of the demethylenated cepharanthine (**5**) over methylene iodide as shown in Table I and found that the best yield of cepharathine (**1**) could be obtained when the three equivalents of the demethylenated cepharanthine (**5**) with respect to methylene iodide was used.

For the synthesis of [methylenedioxy-^{14}C]cepharanthine (**1b**), we prepared [^{14}C]methylene iodide (**3**) from [^{14}C]iodoform[10] which had been derived from barium [2-^{14}C]acetate[9] *via* [1,3-$^{14}C_2$]acetone[9] according to the reported procedure[11] for unlabelled materials. Treatment of the demethylenated cepharanthine (**5**) with [^{14}C]methylene iodide (**3**) in the same manner as with unlabelled methylene iodide provided the desired [methylenedioxy-^{14}C]cepharanthine (**1b**) in good yield.

The synthesis of the demethylenated cepharanthine (**5**) stimulated us to prepare the cepharathine derivative (**4**) having an ethylenedioxy group in the place of the methylenedioxy group in order to examine its biological activities. Treatment of demethylenated cepharanthine (**5**) with 1.1 equivalents of ethylene dibromide according to the above procedure afforded the desired ethylenedioxy product (**4**) in 12.4% yield. The structure of this material was confirmed by the observation that, in the ^{1}H-NMR spectrum, it shows new signals due to an ethylenedioxy group in the region between δ 3.77—4.33.

The results of biological experiments on these materials will be reported elsewhere in the near future.

Experimental

All melting points were measured on a micro melting point hot stage apparatus (Yanagimoto) and are uncorrected. IR spectra were recorded on a Hitachi EPI-G3 spectrometer in nujol. ^{1}H-NMR spectra were recorded on a JEOL JNM-4H-100 spectrometer in deuteriochloroform, with tetramethylsilane as an internal reference. All NH and OH signals were confirmed by their disappearance after addition of deuterium oxide. Mass spectra were measured on a Hitachi RMU-7M spectrometer at 70 eV chamber voltage with a direct inlet system. Optical rotations were determined using a JASCO DIP-140 digital polarimeter. For chromatography (column), Silica Gel 60 (70—230 mesh ASTM), Merck, and aluminium oxide (basic, grade II), Woelm, were used. All identifications of products, except for radioactive ones, were done by IR and TLC comparisons, and melting point determination. Radio-gas chromatography was carried out on a Shimadzu GC-7A equipped with a Aloka RGC-212 radio gas analyzer and TCD radio-detector using a glass column (3 mm × 1.5 m) with 1.5% OV-17. The temperature of injection and detection was 250°C, and the temperature of the column was 80°C. The carrier gas was methane at the flow rate of 50 ml/min. The abbreviations used are as follows: s, singlet; d, doublet; m, multiplet.

Demethylenated Cepharanthine (5)——A solution (9.4 ml) of freshly prepared dry CH_2Cl_2 containing BCl_3 (1.26 g) was added to a solution of cepharanthine (**1**) [benzene·acetone (1: 1) adduct] (1.00 g) in freshly prepared dry CH_2Cl_2 (80 ml) at −40— −35°C with stirring under argon. After 30 min, abs. MeOH (20 ml) was gradually added to the mixture at the same temperature and the whole was evaporated to dryness *in vacuo*. After repeated addition of MeOH and evaporation until evolution of HCl gas ceased, the residue was dissolved in H_2O, made alkaline with 5% $NaHCO_3$ aq., and extracted with Et_2O. The ethereal solution was washed with sat. NaCl aq., dried over $MgSO_4$, and evaporated to dryness *in vacuo*. The residue was chromatographed on SiO_2 with $CHCl_3$. After elution with a mixed solvent [$CHCl_3$: MeOH = 15: 1 (v/v)], the subsequent eluate with a mixed solvent [$CHCl_3$: MeOH = 5: 1 (v/v)] gave a pale yellow amorphous mass[6] (0.59 g). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3480 (OH). ^{1}H-NMR δ: 2.27 and 2.56 (each 3H, s, NCH_3), 2.10—3.30 (12H, m, aliphatic H), 3.57 and 3.87 (each 3H, s, OCH_3), 3.50—4.00 (1H, unclear, C_1- or $C_{1'}$–H), 4.15 (1H, d, J = 7.0 Hz, C_1- or $C_{1'}$–H), 5.44 (1H, s, arom. H), 6.10 (1H, s, arom. H), 6.22—7.40 (10H, m, arom. H and OH × 2). MS m/z: 594 (M^+, 10.6%), 367 (100%), 184 (36.4%).

Methylenation of Demethylenated (±)-Canadine (6) [(±)-Canadine (7)]——A solution of demethylenated (±)-canadine[12] (**6**) (1.01 g) in dry DMF (31 ml) was added to anhydrous cesium fluoride[13] (CsF) (3.29 g) and the mixture was stirred at room temperature for 1 h under argon. After addition of methylene bromide (0.24 ml), the whole was heated at 110—120°C for 3.5 h with stirring, diluted with $CHCl_3$, and filtered. The filtrate was washed with 5% NaOH aq., dried over K_2CO_3, and evaporated to dryness *in vacuo*. Recrystallization of the residue from $CHCl_3$–MeOH gave pale yellow prisms (0.900 g), mp 166—170°C (lit. mp 177—178°C,[14a] mp 172—173°C[14b]).

Methylenation of Demethylenated Cepharanthine (5) [Cepharanthine (1)]——A solution of demethylenated cepharanthine (**5**) (0.170 g: 0.286 mmol) in dry DMF (1.2 ml) was added to CsF[13] (0.302 g: 1.99 mmol). The

mixture was stirred at room temperature for 1 h under argon, then methylene iodide (0.0077 ml: 0.0957 mmol) was added. The stirred mixture was heated at 115—120°C for 2 h. After completion of the reaction, $CHCl_3$ (50 ml) and 5% NaOH aq. (20 ml) were added to the reaction mixture and the aqueous layer was separated. The organic layer was washed with 5% NaOH aq., dried over K_2CO_3, and evaporated to dryness *in vacuo*. The residue was chromatographed on SiO_2 with $CHCl_3$. The eluate with a mixed solvent [$CHCl_3$: MeOH = 15: 1 (v/v)] gave colorless prisms (0.044 g), mp 99—101°C, which were recrystallized from benzene–acetone. *Anal.* Calcd for $C_{37}H_{38}N_2O_6 \cdot C_6H_6 \cdot CH_3COCH_3$: C, 74.37; H, 6.78; N, 3.77. Found: C, 74.23; H, 6.77; N, 3.75. $[\alpha]_D^{19}$ +296° (c=1.00, $CHCl_3$) [lit. $[\alpha]_D^{21}$ +296.6° (c=1.045, $CHCl_3$),[15a] $[\alpha]_D$ +306° (c=0.8, $CHCl_3$)[15b]].

[^{14}C]Methylene Iodide (3)——Sodium arsenite solution (3 ml) [prepared by addition of arsenious oxide (1.00 g) and NaOH (2.00 g) to water (10 ml)] was added to [^{14}C]iodoform[10] (specific activity: 26.4 mCi/mmol) (0.925 g; 62 mCi) which had been prepared from barium [2-^{14}C]acetate according to the reported method.[9] The mixture was heated at 55—60°C for 1 h with stirring under nitrogen. After addition of a further amount of the sodium arsenite solution (1.5 ml), the mixture was heated for 2 h under the same conditions then extracted with Et_2O (20 ml × 3). The ethereal solution was washed with water, dried over a small amount of $CaCl_2$, and carefully concentrated under reduced pressure to give a colorless liquid (0.429 g; 42 mCi) (specific activity: 26.2 mCi/mmol). The radiochemical purity of the material (3) was determined to be 95% by radio-gas chromatography.

[methylenedioxy-^{14}C]Cepharanthine (1b)——A solution of demethylenated cepharanthine (5) (2.90 g: 4.88 mmol) in dry DMF (28 ml) was added to CsF[13] (4.00 g: 26.3 mmol) in a reaction vessel under argon. The mixture was stirred at room temperature for 1 h, a solution of [^{14}C]methylene iodide (specific activity: 26.2 mCi/mmol) [0.429 g; 42 mCi (1.60 mmol)] in dry DMF (8 ml) was added and the whole was heated at 110—120°C for 1 h, then cooled. After addition of 5% NaOH aq., the reaction mixture was extracted with $CHCl_3$ (25 ml × 3). The organic layer was washed with water, dried over K_2CO_3, and evaporated to dryness *in vacuo*. Purification of the residue by column chromatography on SiO_2 with $CHCl_3$–EtOH [12: 1 (v/v)] gave colorless prisms [cepharanthine·benzen·acetone (1: 1: 1) adduct] (0.704 g; 25 mCi), which were recrystallized from benzene–acetone at 5°C.

Radio-chromatographically, this material (specific activity: 26.4 mCi/mmol) showed one radio-peak at *Rf* 0.57, and corresponded to a sample of unlabelled cepharanthine (1), when monitored by TLC[16] on SiO_2 with $CHCl_3$–MeOH [5: 1 (v/v)].

Ethylenation of Demethylenated Cepharanthine (5) [**The Ethylenedioxy Derivative** (4)]——A mixture of demethylenated cepharanthine (5) (0.52 g: 0.874 mmol) and CsF[13] (0.93 g: 6.12 mmol) in dry DMF (3.8 ml) was stirred at room temperature for 1 h under argon. After addition of ethylene dibromide (0.083 ml: 0.960 mmol), the mixture was heated at 110—120°C with stirring. When the reaction was completed, the mixture was diluted with $CHCl_3$ and filtered. The filtrate was washed with 5% NaOH aq., dried over K_2CO_3, and evaporated to dryness *in vacuo*. The residue was dissolved in a minute amount of $CHCl_3$ and a large amount of ether was added to this solution. The resulting precipitate was filtered off and the ethereal solution was evaporated to dryness. The residue was chromatographed on Al_2O_3 with a mixed solvent [benzene: AcOEt = 7: 1 (v/v)]. Elution with the same mixed solvent gave colorless fine needles (0.075 g), mp 140—142°C (dec.), which were recrystallized from CHCl–acetone. *Anal.* Calcd for $C_{38}H_{40}N_2O_6 \cdot 1/2CHCl_3 \cdot 1/2H_2O$: C, 67.07; H, 6.07; N, 4.06. Found: C, 67.12; H, 6.02; N, 3.91. ^{1}H-NMR δ: 2.10—3.77 (13H, m, aliphatic H), 2.57 and 2.61 (each 3H, s, NCH_3), 3.69 and 3.93 (each 3H, s, OCH_3), 3.77—4.03 (2H, unclear, $OC\underline{H}_2CH_2O$), 4.03—4.33 (3H, m, C_1– or $C_{1'}$–H and $OCH_2C\underline{H}_2O$), 5.44 (1H, s, arom. H), 6.23—7.15 (8H, m, arom. H), 7.41 (1H, d, J=8.5 Hz, arom. H). MS m/z: 620 (M^+, 100%), 394 (24.1%), 393 (67.5%), and 197 (50.5%). $[\alpha]_D^{19}$ +341° (c=0.455, $CHCl_3$).

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12) Our demethylenated (±)-canadine, which was prepared according to the reported method,[7b] contains $1/2H_2O$ as water of crystallization. *Anal.* Calcd for $C_{19}H_{21}NO_4 \cdot 1/2H_2O$: C, 67.84; H, 6.59; N, 4.16. Found: C, 68.03; H, 6.39; N, 3.88.
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16) The purity of this material was confirmed by radio-TLC using $CHCl_3$–MeOH [5: 1 (v/v)], $CHCl_3$–MeOH–AcOH [45: 5: 1 (v/v)], hexane–benzene–acetone–AcOEt–MeOH [18: 16: 5: 12: 6 (v/v)], and methyl ethyl ketone saturated with water and by dilution analysis, which indicated 100% purity.