Revised Stereostructure for (+)-Roemecarine and Synthesis of (±)-, (+)-, and (−)-Roemercarine and (±)-Epiroemecarine[1)]

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The stereostructure of (+)-roemecarine, a new 1-benzyl-1,2,3,4-tetrahydroisoquinoline having a hydroxyl group at the 4-position, was confirmed to be 1,4-*trans* by synthesis of (±)-epiroemecarine (1) and (±)-roemecarine (5) *via* *o*-quinol acetates of isocodamine (4). Furthermore, (+)- and (−)-roemecarine (5) were synthesized in good chemical and optical yields by kinetic resolution of (±)-4-*O*-acetyl- (3) or (±)-4,6-*O*,*O*-diacetylroemecarine (7) by the use of immobilized lipase OF-360 (*Candida cylindracea*) in organic solvents. (+)-Roemecarine (5) was proved to have 1*S*,4*S*-configuration.

Keywords (+)-roemecarine; (±)-roemecarine; (±)-epiroemecarine; (−)-roemecarine; (±)-4-*O*-acetylroemecarine; (±)-4,6-*O*,*O*-diacetylroemecarine; kinetic resolution; immobilized lipase OF-360; *Candida cylindracea*; isooctane

Recently, a new 1-benzyl-1,2,3,4-tetrahydroisoquinoline alkaloid having a hydroxyl group at the 4-position, (+)-roemecarine, was isolated from *Roemecaria carica* A. BAYTOP (Papaveraceae) and its structure has been reported to be 1,4-*cis*-1,2,3,4-tetrahydro-7-methoxy-1-(3,4-dimethoxybenzyl)-2-methylisoquinoline-4,6-diol (**1**)[2)] on the basis of proton nuclear magnetic resonance (^{1}H-NMR) spectral evidence. However, the relative stereochemistry of the 1-benzyl and 4-hydroxyl groups did not seem to be clear, because the *syn* relationship proposed for the alkaloid was determined only on the basis of the similarity of the ^{1}H-NMR spectral data to those of 4-hydroxyaporphine, whose conformational rigidity essentially differs from that of 1-benzyltetrahydroisoquinolin-4-ol. Furthermore, as judged from signals due to 8-H in the ^{1}H-NMR spectra[3)] of 1,4-*cis*- (**2**) (δ 6.01) and 1,4-*trans*-4-acetoxy-1-benzyl-1,2,3,4-tetrahydroisoquinolin-6-ols (**3**) (δ 5.84), the upfield-shifted signal (δ 5.76)[2)] reported for (+)-roemecarine strongly suggested that the alkaloid is in the anti relationship. Accordingly, the question of the stereochemistry of the alkaloid could be solved by comparion of the alkaloid with the deacetyl derivative obtained by hydrolysis of 1,4-*cis*- (**2**)[3)] or 1,4-*trans*-4-acetoxytetrahydroisoquinolin-6-ol (**3**),[3)] which can be readily prepared *via* *o*-quinol acetates from isocodamine (**4**). Moreover, optically active roemecarine could be synthesized by means of enzyme-catalyzed kinetic resolution as reported in a preceding paper.[4)] In this paper, we present a revised stereostructure for (+)-roemecarine and describe syntheses of (±)-epiroemecarine (**1**), (±)-roemecarine (**5**), and optically active roemecarine (**5**).

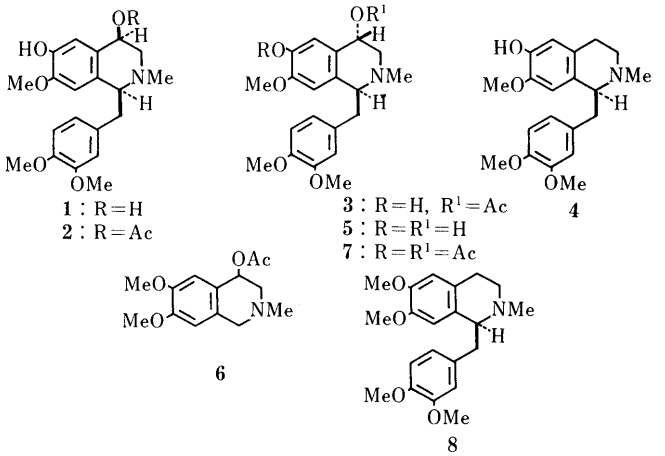

Chart 1

Hydrolysis of (±)-1,4-*cis*-**2**[3)] with 5% methanolic potassium hydroxide at room temperature gave the (±)-1,4-*cis*-diol (**1**) in 59% yield. As shown in Table I, the ^{1}H-NMR spectral data for (±)-**1** were inconsistent with those reported for the alkaloid[2)] as regards the chemical shift (δ 6.53) due to 8-H. On the other hand, the ^{1}H-NMR spectral data for the (±)-1,4-*trans*-diol (**5**) (87% yield) derived from (±)-1,4-*trans*-**3**[3)] in the same manner as described for (±)-**2** were in good accordance with those of the alkaloid described therein. During hydrolysis, isomerization at the 4-position was not observed.

Thus, the stereostructure of (+)-roemecarine was revised to 1,4-*trans*-1,2,3,4-tetrahydro-7-methoxy-1-(3,4-dimethoxybenzyl)-2-methylisoquinoline-4,6-diol and (±)-epiroemecarine (**1**) and (±)-roemecarine (**5**) were synthesized in 3.5% and 30% overall yields, respectively, from (±)-isocodamine (**4**).

Next, synthesis of optically active roemecarine by means of enzyme-catalyzed kinetic resolution was carried out. The optical purity (e.e.) of the products was estimated by

TABLE I. ^{1}H-NMR Spectral Data for (+)-Roemecarine and the (±)-1,4-*trans*-Doil (**5**) and (±)-1,4-*cis*-Diol (**1**)

	(+)-Roemecarine[a)] (**5**)	(±)-1,4-*trans*-Doiol (**5**)	(±)-1,4-*cis*-Diol (**1**)
NMe	2.70	2.69	2.63
7-OMe	3.50	3.48	3.64
3′-OMe	3.78	3.77	3.80
4′-OMe	3.86	3.86	3.80
4-H	4.48 (dd, J=3.2, 2.6)	4.48 (t, J=2.8)	4.33 (br t)
5-H	6.96	6.95	6.95
8-H	5.76	5.73	6.53
2′-H	6.47 (d, J=2)	6.45 (d, J=1.9)	6.70 (d, J=2)
5′-H	6.79 (d, J=8.2)	6.77 (d, J=8.1)	6.70 (d, J=8.3)
6′-H	6.58 (dd, J=8.2, 2)	6.54 (dd, J=8.1, 1.9)	6.54 (dd, J=8.3, 2)

a) Data from ref. 2. Values in parentheses are coupling constants (Hz). Abbreviations: br t, broad triplet; d, doublet; t, triplet; dd, double doublets.

^{1}H-NMR (400 or 500 MHz) spectral analysis as reported in a preceding paper,[4] since reaction of (±)-**5** with *R*-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA Cl)[5] gave the corresponding *R*-(+)-di-MTPA esters, the ^{1}H-NMR spectrum of which showed the distinct peaks (δ 2.37 and 2.53) due to the *N*-methyl protons (see Experimental).

In the present kinetic resolution, benzene or toluene was used as a co-solvent because of the lower solubility of (±)-**3** in isooctane and cyclohexane. Lipases OF-360, MY-30, and C. C. Sigma (*Candida cylindracea*) were used, since reaction with the other lipase, such as Amano A (*Aspergillus niger*), Amano A-6 (*A. niger*), Amano-P (*Pseudomonas* sp.), or R. D. (*Rhizopus delemar*), which was effective in kinetic resolution of (±)-4-acetoxytetrahydroisoquinoline (**6**),[4] was very slow (more than 96 h).

A mixture of (±)-**3** and lipase OF-360 immobilized on Celite in benzene–isooctane (1 : 5) saturated with water was incubated with shaking at 33 °C for 96 h. Usual work-up of the reaction mixture followed by column chromatography gave (−)-4-*O*-acetylroemecarine (**3**) (45%) and (+)-roemecarine (**5**) (44%), $[\alpha]_D^{27}$ +9.23°. The former (**3**) was hydrolyzed in the same manner as noted above to afford (−)-roemecarine (**5**) (57%), $[\alpha]_D^{27}$ −9.18°. Each roemecarine was converted to the corresponding *R*-(+)-di-MTPA ester, the ^{1}H-NMR spectra of which showed optical purity values of 65% for (+)-roemecarine (**5**) and 64% for (−)-roemecarine (**5**). When twice as much Celite and water were used, the optical purity of (−)-**3** was increased (>99% e.e.), although the chemical yield decreased (26%). On the other hand, similar reaction in toluene–cyclohexane (1 : 4) saturated with water proceeded for 72 h to give (+)-**5** (40%; 62% e.e.) and (−)-**3** (39%; 85% e.e.). The results of the reaction in a variety of solvents under similar conditions are listed in Table II.

Thus, (+)- and (−)-roemecarines (**5**) were synthesized in 44% (65% e.e.) and 25% (64% e.e.) overall yields from (±)-**3**, respectively.

In order to improve the optical yield of optically active roemecarines (**5**), reaction in isooctane or cyclohexane without benzene or toluene was chosen, to avoid possible deactivation of the lipase. With this in mind, the (±)-4,6-diacetate (**7**),[3] which is relatively soluble in isooctane or cyclohexane, was used as substrate. Namely, (±)-**7** was incubated with immobilized lipase OF-360 in isooctane saturated with water with shaking at 33 °C for 24 h. Work-up of the reaction mixture as noted above gave (−)-4-*O*-acetylroemecarine (**3**) (35%; 94% e.e.), $[\alpha]_D^{23}$ −74.1°, and (+)-roemecarine (**5**) (48%; 79% e.e.), $[\alpha]_D^{23}$ +12.2°, together with a small amount of **7**, whose optical purity was not determined. A similar reaction in cryclohexane afforded (−)-**7** (27.7%; 11% e.e.), (−)-**3** (34.6%; 98% e.e.), and (+)-**5** (27.5%; 86% e.e.). Although the reaction was a little complicated, the optical yield increased as expected. It is noteworthy that the reaction of the (±)-4,6-diacetate (**7**) gave (−)-**3** together with (+)-**5**.

The absolute configuration of (+)-roemecarine (**5**) was determined as follows. Catalytic hydrogenolysis of (+)-**5** ($[\alpha]_D^{18}$ +11.8°) over 10% palladium on carbon gave isocodamine (**4**). Methylation of **4** with diazomethane in methanol gave *S*-(+)-laudanosine (**8**), mp 91—92 °C ($[\alpha]_D^{21}$ +76.8°), the ^{1}H-NMR spectral data of which were identical with those reported.[6] As the relative configuration between hydroxyl and benzyl groups was *trans*, therefore, the absolute stereostructure of (+)-roemecarine (**5**) was proved to be 1*S*,4*S*-(+)-1,2,3,4-tetrahydro-7-methoxyl-1-(3,4-dimethoxybenzyl)-2-methylisoquinoline-4,6-diol.

In conclusion, the stereostructure of (+)-roemecarine (**5**) was confirmed to be 1,4-*trans*-1,2,3,4-tetrahydro-7-methoxy-1-(3,4-dimethoxybenzyl)-2-methylisoquinoline-4,6-diol by synthesis of (±)-epiroemecarine (**1**) and (±)-roemecarine (**5**) starting from (±)-isocodamine (**4**). Furthermore, (+)- and (−)-roemecarines (**5**) were synthesized in good chemical and optical yields by kinetic resolution of (±)-4-*O*-acetyl- (**3**) or (±)-4,6-*O*,*O*-diacetylroemecarine (**7**) using immobilized lipase OF-360 (*Candida cylindracea*) in organic solvents.

TABLE II. Reaction of (±)-4-*O*-Acetylroemecarine (**3**) with Immobilized Lipase in Organic Solvents

Lipase	Solvent	Reaction time (h)	Chemical yield (%)		Optical purity (% ee)	
			(−)-**3**	(+)-**5**	(−)-**3**	(+)-**5**
OF-360[c]	I-B[a]	96	45	44	64	65
	I-B[b]	96	26	33	>99	55
	I-T	72	48	35	39	62
	C-T	72	39	40	85	62
MY-30[d]	I-B[b]	96	54	45	58	58
C.C. Sigma[e]	I-B[b]	72	43	49	58	40
	C-T	72	70	22	30	43

a) I-B: Isooctane–benzene (5 : 1) was used. *b*) I-B: Isooctane–benzene (4 : 1) was used. I-T: Isooctane–toluene (4 : 1) was used. C-T: Cyclohexane–toluene (4 : 1) was used. *c*) *Candida cylindracea* OF-360 (Meito Sangyo Co., Ltd.). *d*) *C. cylindracea* MY-30 (Meito Sangyo Co., Ltd.). *e*) *C. cylindracea* Sigma (Sigma L 1754).

Experimental

All the melting points were measured on a Büchi 510 melting point measuring apparatus and are uncorrected. ^{1}H-NMR spectra were taken with a JEOL JNM-FX-100, GX-400 or GSX-500 instrument in $CDCl_3$ solution using tetramethylsilane as an internal standard. Mass spectra (MS) were run on a Hitachi RMV-7M or M-80 instrument. Specific rotation was measured on a Perkin Elmer 241 MC or a JEOL DIP-360 polarimeter. Column chromatography was performed on Wakogel C-200 (Wako Pure Chemical Co., Ltd.). Thin layer chromatography (TLC) and preparative TLC (PTLC) were carried out on Kieselgel $60F_{254}$ (Merck) and F_{254} (Merck). Low pressure liquid chromatography (LPLC) was performed by a Kusano Kagakukeiki machine equipped with a KP-6H micro pump using a glass column packed with silica gel.

Preparation of (±)-Epiroemecarine (1) and (±)-Roemecarine (5) A diastereomeric mixture (106.2 mg) of monoacetates readily prepared *via* the *o*-quinol acetates from (±)-**4** (200 mg) according to the method reported previously[3] was treated with LPLC (eluting solvent: C_6H_6–EtOAc–MeOH, 24 : 16 : 1) to give (±)-**2** (13.2 mg, 5.7%) and (±)-**3** (81.3 mg, 35%), each as an oil. (±)-**1**: A solution of (±)-**2** (28.1 mg) in 5% KOH–MeOH (6 ml) was stirred at room temperature for 4 h. Usual work-up of the reaction mixture gave an oily product, which was purified by PTLC (developing solvent: $CHCl_3$–MeOH, 10 : 1) to afford (±)-**1** (14.7 mg, 59%) as an oil. It was crystallized from ether. mp 140—141.5 °C (C_6H_6–hexane). High-resolution MS (*m/z*) Calcd for $C_{20}H_{24}NO_5$ $(M-1)^+$: 358.1648. Found: 358.1656. (±)-**5**: Treatment of (±)-**3** (45 mg) with 5% KOH–MeOH (10 ml) as noted for (±)-**2** for 6 h followed by PTLC (developing solvent: $CHCl_3$–MeOH, 10 : 1) gave (±)-**5** (34 mg, 59%) as an oil, which was crystallized from ether. mp 115.5—117 °C (C_6H_6–hexane). *Anal.* Calcd for $C_{20}H_{25}NO_5 \cdot 0.5H_2O$: C, 65.15; H, 7.06; N, 3.80. Found: C, 65.21; H, 6.80; N, 3.87. The ^{1}H-NMR (400 MHz) spectral data for (±)-**1** and (±)-**5** are shown in Table I. Acetylation of (±)-**1** or (±)-**5** with Ac_2O–pyridine at room temperature gave the (±)-1,4-*cis*- or (±)-1,4-*trans*-diacetate, the ^{1}H-NMR spectrum of which

was identical with that reported previously.[3]

Preparation of (+)- and (−)-Roemecarines (5) From (±)-**3**: (a) A mixture of (±)-**3**[3] (100 mg) and lipase OF-360 immobilized on Celite, prepared from lipase (100 mg), Celite 535 (300 mg) and water (0.2 ml) according to the method[14] reported in a preceding paper, was incubated with shaking in benzene (10 ml)–isooctane (40 ml) saturated with water at 33 °C for 72 h, and then the same amounts of immobilized lipase and isooctane (10 ml) were further added to the reaction mixture. Incubation was continued at the same temperature for an additional 24 h. The catalyst was filtered off and washed with a mixture of $CHCl_3$ and MeOH. The combined filtrate was concentrated to dryness *in vacuo* to afford an oily product, which was subjected to column chromatography. Elution with $CHCl_3$–MeOH (100 : 1) gave (−)-**3** (44.7 mg, 45%) and (+)-roemecarine (**5**) (39.0 mg, 44%; 65% e.e.), $[\alpha]_D^{27}$ +9.23° (*c*=2.2, MeOH) [lit.[2] $[\alpha]_D$ +9° (*c*=0.07, MeOH)]. The former was hydrolyzed in the same manner as noted for (±)-**3** to give (−)-roemecarine (**5**) (22.6 mg, 57%; 64% e.e.), $[\alpha]_D^{27}$ −9.18° (*c*=2.2, MeOH). The ^{1}H-NMR spectra of (+)- and (−)-**5** were identical as noted as below. The optical purity (e.e.) of each product was estimated on the basis of the ratio of NMe proton signals [δ 2.37 for 1*S*,4*S*-(+)-**5**-(+)-di-MTPA; δ 2.53 for 1*R*,4*R*-(−)-**5**-(+)-di-MTPA] in the ^{1}H-NMR (400 or 500 MHz) spectrum of the (+)-di-MTPA ester of each product. Assignment of the NMe proton signals was performed by comparison of the ^{1}H-NMR spectrum of the (+)-di-MTPA ester of 1*R*,4*R*-(−)-**5** with that of 1*S*,4*S*-(+)-**5**, the absolute configuration which was determined as described below.

(b) A mixture of (±)-**3** (100 mg) and immobilized lipase [lipase (100 mg), Celite 535 (600 mg) and water (0.4 ml)] in benzene–isooctane (1 : 4) (50 ml) was incubated with stirring at 33 °C for 96 h. Work-up as noted in (a) [except elution with $CHCl_3$–MeOH (100 : 1 and 20 : 1)] of the reaction mixture gave (−)-**3** [25.6 mg, 26%; >99% e.e.; $[\alpha]_D^{27}$ −95.5° (*c*=1.70, $CHCl_3$), MS *m/z*: 401 (M^+); ^{1}H-NMR (100 MHz) δ: 2.07 (3H, s, OAc), 2.63 (3H, s, N-Me), 3.52 (3H, s, 7-OMe), 3.76, 3.83 (6H, each s, 2×OMe), 5.74 (1H, dd, *J*=2, 4 Hz, 4-H), 5.82 (1H, s, 8-H)] and (+)-**5** [29.3 mg, 33%; 55% e.e.; $[\alpha]_D^{27}$ +8.5° (*c*=1.95, MeOH); MS *m/z*: 358 (M^+); ^{1}H-NMR (100 MHz) δ: 2.64 (3H, s, NMe), 3.45 (3H, s, 7-OMe), 3.74, 3.83 (6H, each s, 2×OMe), 4.44 (1H, t, *J*=3 Hz, 4-H), 5.68 (1H, s, 8-H)].

(c) Reaction of (±)-**3** (100 mg) with immobilized lipase [lipase (100 mg), Celite 535 (300 mg), and water (0.2 ml) in a variety of solvents (50 ml) was carried outunder similar conditions to those as described in (b). The results are listed in Table II.

From (±)-**7**: (a) A mixture of (±)-**7**[3] (100 mg) and immobilized lipase [lipase (100 mg), Celite 535 (300 mg) and water (0.4 ml)] in isooctane (40 ml) saturated with water was incubated with shaking at 33 °C for 24 h. Work-up of the reaction mixture in the same manner as noted above gave **7** (3%), (−)-**3** [31.7 mg, 35%; 94% e.e.; $[\alpha]_D^{23}$ −74.1° (*c*=2.08, $CHCl_3$)], and (+)-**5** [39.0 mg, 48%; 79% e.e.); $[\alpha]_D^{28}$ +12.2° (*c*=1.65, MeOH)], respectively. The ^{1}H-NMR spectra of **7**, (−)-**3**, and (+)-**5** were identical with those of authentic specimens. The optical purity of the products was estimated in the same manner as noted above.

(b) Compound (±)-**7** (100 mg) was incubated in cyclohexane (40 ml) saturated with water under similar conditions to those noted above to give (−)-**7** [27.7 mg, 27.7%; 11% e.e.; $[\alpha]_D^{26}$ −11.2° (*c*=1.61, $CHCl_3$)], (−)-**3** [31.3 mg, 34.6%; 98% e.e.; $[\alpha]_D^{26}$ −79.1° (*c*=1.85, $CHCl_3$)], and (+)-**5** [22.3 mg, 27.5%; 86% e.e.; $[\alpha]_D^{26}$ +13.5° (*c*=1.17, MeOH)].

Absolute Configuration of (+)-Roemecarine (5) A mixture of (+)-**5** (35.8 mg), [$[\alpha]_D^{18}$ +11.8° (*c*=1.26, MeOH)] and 10% Pd–C (12 mg) in dioxane (6 ml) and concentrated HCl (0.3 ml) was shaken with H_2 (30 p.s.i.) at room temperature for 6 h. Usual work-up of the reaction mixture gave isocodamine (**4**) (29.8 mg, 86.9%), which was methylated with CH_2N_2–ether (4 ml) in MeOH (4 ml) at room temperature for 46 h. Usual work-up of the reaction mixture followed by purification by PTLC (developing solvent: $CHCl_3$–MeOH, 20 : 1) gave *S*-(+)-laudanosine (**8**) (15.3 mg, 49.9%), mp 91—92 °C, $[\alpha]_D^{21}$ +76.8° (*c*=0.60, EtOH) [lit.[6] $[\alpha]_D^{22}$ +78.9° (*c*=1.55, EtOH)]. High-resolution MS (*m/z*) Calcd for $C_{21}H_{27}NO_4$ (M^+): 357.1940. Found; 357.1946. The ^{1}H-NMR spectral datum was identical with that reported.[6]

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References

1) A part of this work has been published in: O. Hoshino, K. Itoh, B. Umezawa, H. Akita, and T. Oishi, *Heterocycles*, **26**, 2099 (1987).
2) T. Gözler, B. Gözler, N. Tanker, A. J. Freyer, H. Guinaudeau, and M. Shamma, *Hetercycles*, **24**, 1227 (1986).
3) O. Hoshino, M. Ohtani, B. Umezawa, and Y. Iitaka, *Chem. Pharm. Bull.*, **32**, 4873 (1984).
4) O. Hoshino, K. Itoh, B. Umezawa, H. Akita, and T. Oishi, *Tetrahedron Lett.*, **29**, 567 (1988); *idem*, *Chem. Pharm. Bull.*, **38**, 3273 (1990).
5) J. A. Dale and H. S. Mosher, *J. Am. Chem. Soc.*, **95**, 512 (1973).
6) Z. Czarmocki, D. B. MacLean, and W. A. Szarek, *Can. J. Chem.*, **64**, 2205 (1986).