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Isolation of Clivacetine from *Clivia miniata* Regel. (Amaryllidaceae)

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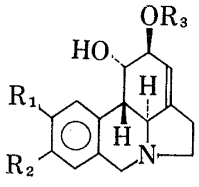
Clivia miniata Regel. (Amaryllidaceae) was found to contain a novel alkaloid, clivacetine (**2**), as well as lycorine (**3**), clivatine (**4**), clivimine (**5**), and clivonine (**6**). Clivacetine was identified as O-acetoacetylclivonine (**2**) and has an interesting structure from a biogenetic viewpoint, since it is a possible biosynthetic precursor of clivimine (**5**).

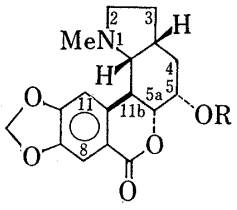
Keywords——*Clivia miniata* Regel.; clivacetine; clivatine; clivimine; clivonine; lycorine; Amaryllidaceae

Previously we reported[2] the isolation of carinatine (**1**) from the bulbs of *Zephyranthes carinate* Herb. (Amaryllidaceae).

This paper describes the isolation of a novel alkaloid clivacetine (**2**), as well as lycorine (**3**), clivatine (**4**),[3] clivimine (**5**), and clivonine (**6**), from the rhizomes or the whole plant of *Clivia miniata* Regel[4,5] (Japanese name, Akabanakunshiran) and the structural assignment of **2**.



1: R_1=OH, R_2=OMe, R_3=Me
3: R_1,R_2=OCH_2O, R_3=H



2: R=$-COCH_2COCH_3$
4: R=$-COCH_2CH(OH)CH_3$
6: R=H
7: R=$-COCH_3$
8: R=$-COCH_2CH(OAc)CH_3$

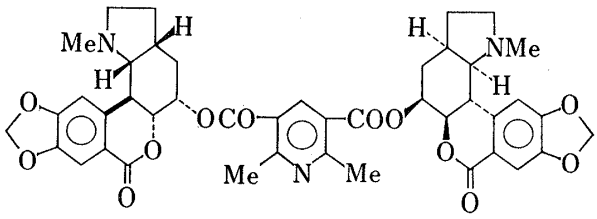


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Chart 1

Crude basic material was extracted from fresh rhizomes of *C. miniata* Regel. by the method of Wildman and Bailey.[6] Lycorine (**3**) was isolated from a chloroform solution of the crude material, utilizing its low solubility in this solvent, and was identified by direct comparison with an authentic sample of **3**. The chloroform solution was subjected to preparative thin-layer chromatography (PLC) using silica gel–chloroform–methanol to give two

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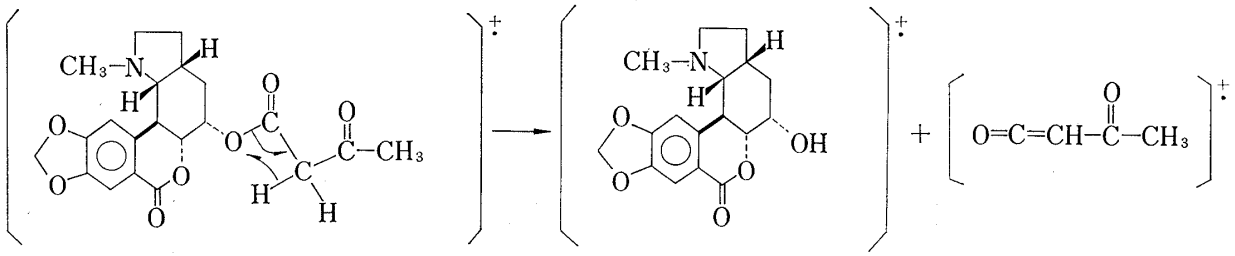
2) S. Kobayashi, H. Ishikawa, M. Kihara, T. Shingu, and T. Hashimoto, *Chem. Pharm. Bull.*, **25**, 2244 (1977).
3) W. Dŏpke, *Heterocycles*, **6**, 551 (1977).
4) C.K. Briggs, P.F. Highet, R.J. Highet, and W.C. Wildman, *J. Am. Chem. Soc.*, **78**, 2899 (1956).
5) H.-G. Boit and B. Mehlis, *Naturwissenschaften*, **48**, 603 (1961).
6) W.C. Wildman and D.T. Bailey, *J. Org. Chem.*, 33, 3749 (1968).

fractions, *Rf* 0.78—0.53 and *Rf* 0.52—0.43: the first fraction gave clivimine (**5**) and clivacetine (**2**), and the second, clivatine (**4**). In the second run, clivonine (**6**) and the alkaloids **2**—**5** were isolated from the fresh whole plant (leaves and rhizomes).

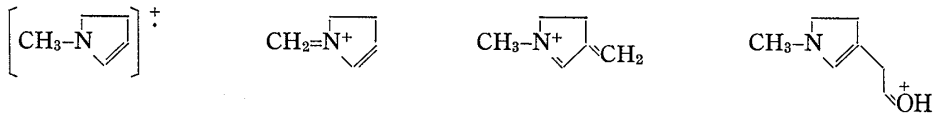
Clivimine (**5**),[5,7] mp 258—259° (dec.) and clivonine (**6**),[4,7] mp 193—194°, were identified from their elemental analyses and spectral data, and by conversion[8] of **5** to **6**.



2
$C_{21}H_{23}NO_7$
m/e 401 (6.7%)
Calcd: 401.1473
Found: 401.1467

6
$C_{17}H_{19}NO_5$
m/e 317 (55.7%)
Calcd: 317.1263
Found: 317.1268

$C_4H_4O_2$
m/e 84 (10.3%)
Calcd: 84.0212
Found: 84.0252



C_5H_9N
m/e 83 (base peak)
Calcd: 83.0735
Found: 83.0776

C_5H_8N
m/e 82 (23.2%)
Calcd: 82.0657
Found: 82.0698

$C_6H_{10}N$
m/e 96 (63.1%)
Calcd: 96.0814
Found: 96.0816

$C_7H_{12}NO$
m/e 126 (1.4%)
Calcd: 126.0919
Found: 126.0922

Chart 2. Mass Spectral Fragments of Clivacetine (**2**)

The novel alkaloid clivacetine (**2**), mp 152—155°, $C_{21}H_{23}NO_7$, has $[\alpha]_D^{24}$ +53.8° (c=0.67, chloroform). The base (**2**) appeared to be a clivonine-type alkaloid on the basis of its high-resolution mass spectrum (MS) [*m/e* 401 (M^+)], since the characteristic and diagnostic fragment ion peaks[7,9] of clivonine-type alkaloids appeared at *m/e* 83 (the base peak, C_5H_9N), 96 (the second largest peak, $C_6H_{10}N$), 82 (C_5H_8N), and 126 ($C_7H_{12}NO$) (see Chart 2). The ultraviolet (UV) spectrum, with λ_{max}^{MeOH} (log. ε) 205 (4.27), 225 (4.38), 266 (3.89), and 306 (3.81), and the optical rotatory dispersion (ORD) curve were very similar to those of **6**, as shown in Fig. 1. The infrared (IR) spectrum of the base showed carbonyl absorptions at 1740 and 1720 cm^{-1}. As shown in Table I, the nuclear magnetic resonance (NMR) spectrum of the base (**2**) was very similar to that[7,10] of O-acetylclivonine (**7**), except that a methylene singlet (2H) was present at δ 3.49. The singlet (δ 3.49) was assignable to an active methylene function such as a –OCO–CH_2CO–, since it disappeared upon exchange with deuterium oxide, and a singlet at δ 2.28 (3H) showed the presence of a –$COCH_3$ group. This means that clivacetine has the partial formula, –OCO–CH_2COCH_3. On the basis of these data, the structure of clivacetine was assigned as **2**. This assignment is consistent with the finding that the mass spectrum (see Chart 2) of **2** showed the molecular ion peak of **6** at *m/e* 317 and the acetylketene ion peak ($C_4H_4O_2$) at *m/e* 84. Finally, the structure of **2** was confirmed by conversion of **2** to O-acetylclivatine (**8**) as described below.

7) W. Döpke, M. Bienert, A.L. Burlingame, H.K. Schnoes, P.W. Jeffs, and D.S. Farrier, *Tetrahedron Lett.*, **1967**, 451.
8) B. Mehlis, *Naturwissenschaften*, **52**, 33 (1965).
9) H.K. Schnoes, D.H. Smith, A.L. Burlingame, P.W. Jeffs, and W. Döpke, *Tetrahedron*, **24**, 2825 (1968).
10) P.W. Jeffs, J.F. Hansen, W. Döpke, and M. Bienert, *Tetrahedron*, **27**, 5065 (1971).

Döpke reported[3] that clivatine was an ester (**4**) of clivcnine (**6**) and β-hydroxybutyric acid, but gave no details about the presence of the β-hydroxybutyryl group in **4**. Therefore, we carried out further investigations on the structure of clivatine (**4**), mp 159—161°, $C_{21}H_{25}NO_7$. The base (**4**) was suggested to be a clivonine-type alkaloid by its MS [*m/e* 403 (M^+), 83, 96, 82, and 126], as described for **2**. The UV spectrum and the ORD curve of **4** were very similar to those of **6** (see Fig. 1). The IR spectrum of **4** showed carbonyl absorptions at 1730 and 1700 cm^{-1}, and a hydroxyl absorption at 3600—3000 cm^{-1}. In the NMR spectrum of **4** in $CDCl_3$, monitoring the higher line (δ 1.17) of a doublet (C-4′ H_3) gave INDOR signals around δ 4.20 ascribable to C-3′-H. Decoupling of the signals of C-3′-H (which overlap those of C-5a-H) by irradiation at δ 4.20 reduced the doublet (C-4′ H_3) to a singlet. In $CDCl_3$–C_6D_6 (1: 1), the overlaps of the signals (C-5a-H and C-3′-H) are resolved to a double doublet at δ 3.79, assignable to C-5a-H, and a sextet at δ 4.09, assignable to C-3′-H. Monitoring the lowest line (δ 4.27) of C-3′-H gave INDOR peaks at δ 1.21 (C-4′ H_3) and around δ 2.36 (C-2′ H_2). These data indicate that clviatine has the partial formula $-COCH_2CH(OH)CH_3$ and the structure **4**.

This was supported by the conversion of **4** to O-acetylclivatine (**8**): treatment of **4** with acetic anhydride and pyridine at room temperature gave **8**, mp 142—145°, $C_{23}H_{27}NO_8$, $[\alpha]_D^{20}$

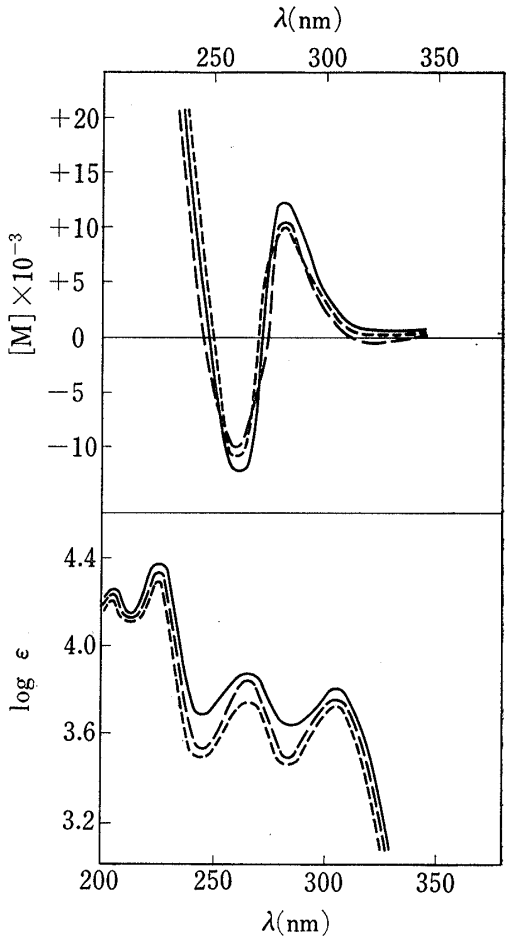


Fig. 1. ORD and UV Spectra in MeOH: Clivacetine (**2**) (——); Clivatine (**4**) (– – –); Clivonine (**6**) (-----)

Table I. Chemical Shifts[a] of Clivacetine (**2**), Clivatine (**4**), and O-Acetylclivonine (**7**) ($CDCl_3$, δ)

Compd. No.	C-11-H	C-8-H	C-5-H	C-5a-H	C-11b-H	C-11c-H
2	7.81	7.45	5.42(q)[b]	4.17(dd)[c]	3.20(m)	2.96(m)
4	7.70	7.45	5.40(m)	4.18(dd)[c]	3.27(m)	2.71(m)
7[f]	7.78	7.45	5.35	4.10(dd)[e]	3.18	2.95(m)

Compd. No.	C-2′ H_2 or H_3	C-3′-H	C-4′ H_3	OCH_2O	NCH_3
2	3.49		2.28	6.03	2.56
4	2.20(m)	4.20(m)[d]	1.21(d)[d]	6.03	2.55
7[f]	2.09			6.00	2.55

a) Signals are singlets unless otherwise indicated (in parentheses).
b) q-like, $J_{5-5a}=J_{5-4\alpha}=J_{5-4\beta}=3$ Hz.
c) $J_{5a-11b}=12$, $J_{5a-5}=3$ Hz.
d) $J_{4'-3'}=6.5$ Hz.
e) $J_{5a-11b}=12.5$, $J_{5a-5}=3$ Hz.
f) See refs. 7 and 10.

+29.2° (c=0.72, chloroform). In the NMR spectrum of **8**, the signal (δ 5.22) of C-3′-H was 1.02 ppm downfield from that (δ 4.20) of **4** and the acetyl protons appeared at δ 2.01 as a singlet.

O-Acetylclivatine (**8**) was also obtained from clivacetine (**2**) as follows: reduction of **2** with sodium borohydride gave a product as white prisms, mp 154—157°, $[\alpha]_D^{22}$ +33.2° (chloroform), which is less than the specific rotation of **4**. The melting point of this compound was not depressed on admixture with an authentic sample of **4**, and its IR and NMR spectra were very similar to, but not identical with those of **4**. Treatment of this compound with acetic anhydride and pyridine afforded white needles, mp 136—141°, with $[\alpha]_D^{20}$ +28.1° (chloroform), and this material was found to be identical with an authentic sample of **8** by direct comparison of their IR, NMR, and ORD spectra and by the mixed melting point test.

Clivacetine (**2**), a novel alkaloid having an acetoacetoxyl function, is important from a biosynthetic point of view, since **2** is a possible biosynthetic precursor of clivimine (**5**).[11]

Experimental

All melting points are given as uncorrected values. The spectrophotometers used were a Hitachi EPI-G2 for IR spectra, a Shimadzu UV-200 for UV spectra, a Hitachi RMU-6C or a JEOL JMS-D 300 for MS, a Yanagimoto OR-50 for optical rotations, a JASCO ORD/UV-5 for ORD spectra, and a JEOL JNM-PS-100 or a Hitachi R-22 for NMR spectra using TMS as an internal standard. The abbreviations used are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. The plates used for PLC were coated with silica gel (Kieselgel, PF_{254} Merk).

Isolation of Alkaloids from *C. miniata* Regel.——Following the method of Wildman and Bailey,[6] fresh rhizomes (1.8 kg) of this plant were ground in 99% EtOH in a mixer. The insoluble material was extracted four times with 2.7 liters of 99% EtOH. The ethanolic extract was evaporated down to approximately 0.9 liter *in vacuo*, made acidic (pH 4) with tartaric acid, and washed with ether until the ether layer was colorless to remove neutral and acidic materials. The aqueous acidic solution was made basic (pH 8) with conc. NH_4OH and extracted eight times with 200 ml portions of $CHCl_3$. The extract was concentrated *in vacuo* to give crude alkaloids (1.45 g, 0.081%). The crude alkaloids gave $CHCl_3$-insoluble (304 mg) and $CHCl_3$-soluble (1015 mg) materials when mixed with $CHCl_3$.

The $CHCl_3$-insoluble material gave **3** (270 mg, 0.015% yield). The $CHCl_3$-soluble material was subjected to PLC using SiO_2-[$CHCl_3$–MeOH (10: 1)] to give two fractions: I, *Rf* 0.78—0.53 (386 mg), and II, *Rf* 0.52—0.43 (127 mg). Elution of fraction I with $CHCl_3$–MeOH (1: 1) gave crude bases, which were subjected to PLC using SiO_2-[MeOH–ethyl acetate (1: 5)] to afford two fractions, *Rf* 0.89—0.81 and *Rf* 0.81—0.64. The former fraction gave **5** (51 mg, 0.0028% yield) and the latter gave **2** (39 mg, 0.0022% yield). Elution of fraction II with $CHCl_3$–MeOH (1: 1) gave **4** (32 mg, 0.0018% yield) after purification by PLC using SiO_2-[$CHCl_3$–diethylamine (10: 1)].

Similar extraction of the fresh whole plant (rhizomes and leaves) (8.3 kg) gave crude bases (11.38 g, 0.137% yield), from which the $CHCl_3$-soluble material (8.47 g) and the $CHCl_3$-insoluble material (3) (2.9 g) were obtained. The former material (8.47 g) was subjected to PLC using SiO_2-[$CHCl_3$–ethyl acetate (3: 5)] to give three fractions: I, *Rf* 0.67—0.56 (1.50 g); II, *Rf* 0.56—0.46 (0.96 g); III, *Rf* 0.36—0.22 (1.60 g). Each fraction was eluted with $CHCl_3$–MeOH (1: 1). Fraction I was triturated with ethyl acetate to give **5** (1.26 g). Fraction II was subjected to PLC using SiO_2-[$CHCl_3$–diethylamine (5: 1)] to give three fractions: II-A, *Rf* 0.93—0.73 (547 mg); II-B, *Rf* 0.73—0.66 (93 mg); II-C, *Rf* 0.66—0.48 (292 mg). Fraction II-A was triturated with ethyl acetate to give additional **5** (302 mg, total 1.565 g, 0.0189% yield), and Fraction II-B was triturated with ethyl acetate to give **6** (56 mg). Fraction II-C gave clivacetine (**2**) (76 mg, 0.0009% yield) after recrystallization from ethyl acetate. Fraction III was purified by PLC using SiO_2-[$CHCl_3$–MeOH (10: 1)] to give a material (758 mg, *Rf* 0.65—0.41), which was subjected to PLC using SiO_2-[CH_2Cl_2–MeOH (10: 1)] to afford two fractions: III-A, *Rf* 0.55—0.43 (329 mg) and III-B, *Rf* 0.43—0.32 (263 mg). Fraction III-A gave **4** (248 mg, 0.0031% yield) after recrystallization from benzene, and fraction III-B gave additional **6** (182 mg, total 238 mg, 0.0029% yield).

Lycorine (**3**)——Crude base **3** was recrystallized from EtOH as white prisms, mp 235—238° (dec.), undepressed on admixture with an authentic sample of **3**. *Anal.* Calcd for $C_{16}H_{17}NO_4$: C, 66.88; H, 5.96; N, 4.88. Found: C, 66.64; H, 5.98; N, 4.75. Its hydrochloride, mp 229—230° (dec.) (from EtOH). *Anal.* Calcd for $C_{16}H_{17}NO_4$·HCl: C, 59.35; H, 5.60; N, 4.33. Found: C, 59.34; H, 5.63; N, 4.12.

Clivimine (**5**)——Crude **5** was crystallized from ethyl acetate as white prisms, mp 258—259° (dec.) [lit.[5] mp 264—266° (dec.)]. $[\alpha]_D^{24}$ +26.9° (c=0.67, $CHCl_3$), [lit.[5] $[\alpha]_D$ +32° ($CHCl_3$)]. IR ν_{max}^{KBr} cm^{-1}: 1720 (CO), 940 (OCH_2O). NMR ($CDCl_3$, δ): 8.53 (1H, s, γ-H in the pyridine ring), 7.52 (2H, d, J_{11-11b}=1 Hz, 2×C-11-H),

11) A biogenetic-type synthesis of **5** from **2** will be reported elsewhere.

7.44 (2H, s, 2×C-8-H), 6.09 and 6.06 (each 2H, d, $J=2$ Hz, AB-type of $2\times OCH_2O$), 5.57 (2H, m, 2×C-5-H), 4.20 (2H, dd, $J_{5a-11b}=12$, $J_{5a-5}=3.5$ Hz, 2×C-5a-H), 3.06 (2H, m, $J_{11b-5a}=12$ Hz, 2×C-11b-H), 2.87 (6H, s, $2\times\alpha$-CH_3 in the pyridine ring), 2.34 (6H, s, $2\times NCH_3$). *Anal.* Calcd for $C_{43}H_{43}N_3O_{12}\cdot 1/2H_2O$: C, 64.34; H, 5.53; N, 5.24. Found: C, 64.23; H, 5.43; N, 5.45.

Clivonine (6)——Crude **6** was recrystallized from ethyl acetate as white prisms, mp 193—194° (lit.[4] mp 199—200°), $[\alpha]_D^{24}$ +46.9° ($c=0.68$, $CHCl_3$), [lit.[4] $[\alpha]_D^{23}$ +41.2° ($CHCl_3$)]. MS *m/e*: 317 (M^+). UV λ_{max}^{MeOH} nm, (log ε): 204 (4.21), 225 (4.31), 266 (3.75), 305 (3.75). IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1680 (CO), 930 (OCH_2O). NMR ($CDCl_3$, δ): 7.73 (1H, d, $J_{11-11b}=1$ Hz, C-11-H), 7.43 (1H, s, C-8-H), 6.00 (2H, diffuse s, OCH_2O), 4.24 (1H, q-like, $J_{5-5a}=J_{5-4\alpha}=J_{5-4\beta}=3$ Hz, C-5-H), 4.08 (1H, dd, $J_{5a-11b}=12$, $J_{5a-5}=3$ Hz, C-5a-H), 3.23 (1H, dd, $J_{11b-5a}=12$, $J_{11b-11c}=10$ Hz, C-11b-H), 2.87 (1H, dd, $J_{11c-11b}=10$, $J_{11c-3a}=6$ Hz, C-11c-H), 2.52 (3H, s, NCH_3). ORD ($c=0.012$, MeOH) $[M]^{24}$ (nm): +1360° (310), +10300° (279) (peak), 0° (270), −10900° (259) (trough), 0° (250), +17100° (240). *Anal.* Calcd for $C_{17}H_{19}NO_5$: C, 64.34; H, 6.04; N, 4.41. Found: C, 64.05; H, 6.05; N, 4.28.

Conversion of Clivimine (5) to Clivonine (6)——According to Mehlis,[8] clivonine (**6**) (111 mg, 69.4%), mp 192—193°, was obtained from clivimine (**5**) (200 mg). This product was identical with an authentic sample of **6** by direct comparison of their IR and ORD spectra and by the mixed melting point test.

Clivacetine (2)——Crude **2** was recrystallized from ethyl acetate as white prisms, mp 152—155°. ORD ($c=0.012$, MeOH) $[M]^{24}$ (nm): +1600° (310), +12400° (281) (peak), 0° (272), −12600° (264) (trough), 0° (249), +14700° (240). *Anal.* Calcd for $C_{21}H_{23}NO_7$: C, 62.83; H, 5.78; N, 3.49. Found: C, 62.70; H, 5.84; N, 3.34. No signals of an enol-form were detectable in the NMR spectrum (in $CDCl_3$) of **2**.

Clivatine (4)——Crude **4** was recrystallized from ethyl acetate as white prisms, mp 159—161°, (lit.[5] mp 166—169°). $[\alpha]_D^{24}$ +47.0° ($c=0.68$, $CHCl_3$), [lit.[5] $[\alpha]_D^{25}$ +52° ($CHCl_3$)]. UV λ_{max}^{MeOH} nm (log. ε): 205 (4.23), 225 (4.33), 266 (3.76), 306 (3.75). ORD ($c=0.011$, MeOH) $[M]^{20}$ (nm): 0° (338), −500° (320) (trough), 0° (313), +10400° (279) (peak), 0° (271), −10800° (260) (trough), 0° (248), +10700° (240). *Anal.* Calcd for $C_{21}H_{25}NO_7$: C, 62.52; H, 6.25; N, 3.47. Found: C, 62.32; H, 6.30; N, 3.37.

O-Acetylclivatine (8)——(i) From Clivatine (**4**): A mixture of **4** (50 mg), acetic anhydride (2 ml), and pyridine (2 ml) was allowed to stand at room temperature for two days, and then concentrated under reduced pressure to give a brown oil (72 mg). The oil was triturated with ethyl acetate to give **8** (38 mg) as white needles, mp 142—145° (from ethyl acetate). UV λ_{max}^{MeOH} nm (log. ε): 205 (4.35), 225 (4.45), 266 (3.88), 306 (3.88). IR ν_{max}^{KBr} cm^{-1}: 1740, 1710 (CO), 940 (OCH_2O). NMR ($CDCl_3$, δ): 7.84 (1H, s, C-11-H), 7.48 (1H, s, C-8-H), 6.02 (2H, s, OCH_2O), 5.26 (2H, m, C-5-H and C-3′-H), 4.14 (1H, dd, $J_{5a-11b}=12$, $J_{5a-5}=3$ Hz, C-5a-H), 2.56 (3H, s, NCH_3), 2.01 (3H, s, $COCH_3$), 1.29 (3H, d, $J=6$ Hz, C-4′ H_3). ORD ($c=0.009$, MeOH) $[M]^{20}$ (nm): 0° (312), +14700° (279) (peak), 0° (271), −14500° (259) (trough), 0° (248), +23500° (236). *Anal.* Calcd for $C_{23}H_{27}NO_8$: C, 62.01; H, 6.11; N, 3.14. Found: C, 61.80; H, 6.10; N, 2.96.

(ii) From Clivacetine (**2**): A mixture of **2** (46 mg), conc. HCl (0.02 ml), and MeOH (10 ml) was concentrated *in vacuo*. A solution of the resulting residue in MeOH (10 ml) was treated with $NaBH_4$ (43 mg) at −15° for 50 min. After addition of acetic acid (0.5 ml), the mixture was concentrated *in vacuo* to give a residue, which was subjected to PLC using SiO_2–[$CHCl_3$–diethylamine (10: 1)]. Elution of the material of *Rf* 0.71—0.60 with $CHCl_3$–MeOH (1: 1) gave a pale yellow oil (40 mg). The oil was triturated with benzene to give white prisms (31 mg), mp 154—157°. *Anal.* Calcd for $C_{21}H_{25}NO_7\cdot 1/4H_2O$: C, 61.86; H, 6.31; N, 3.44. Found: C, 62.00; H, 6.28; N, 3.72. Similar treatment of this compound (37 mg) with acetic anhydride (2 ml) and pyridine (2 ml) gave an oily crude product (38 mg), which was triturated with ethyl acetate to afford two compounds, white needles (15 mg), mp 136—141°, and white prisms (5 mg), mp 137—154°. The former compound was found to be identical with an authentic sample of **8** by direct comparison of their IR, NMR, and ORD spectra and by the mixed melting point test. Found: C, 61.80; H, 6.15; N, 3.08.

The melting point of the latter compound was clearly depressed on admixture with **8**.

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