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(+)-CORYNOLINE 11-*O*-SULFATE FROM *CORYDALIS INCISA**

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Key Word Index—*Corydalis incisa*; Papaveraceae; alkaloid; (+)-corynoline 11-*O*-sulfate; hydrobenzo[*c*]phenanthridine.

Abstract—A novel *O*-sulfated alkaloid isolated from *Corydalis incisa* was characterized as the 11-*O*-sulfate of (+)-corynoline.

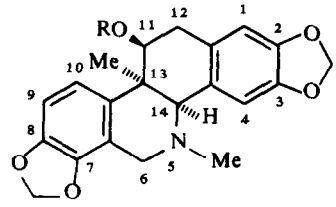
INTRODUCTION

The alkaloidal components of *Corydalis incisa* Pers (Papaveraceae) have been studied by several groups of workers [1–3] and found to be very rich in hydrobenzo-[*c*]phenanthridine-type alkaloids. Amongst them corynoline, the main alkaloid in this plant, has been isolated as a racemate. Recent careful examination in our laboratory, however, resulted in the isolation of optically active (+)-corynoline from the same source [4]. This paper deals with the structural elucidation of an optically active corynoline homologue which was tentatively named as TN-20 [2].

RESULTS AND DISCUSSION

The tertiary non-phenolic alkaloid fraction, obtained by the previously described method [2], was chromatographed on Si gel and successive elution with hexane–EtOAc (from 4:1 to 100% EtOAc), methyl ethyl ketone and methyl ethyl ketone saturated with water. The fraction eluted with methyl ethyl ketone was further purified by chromatography on Si gel with $CHCl_3$–MeOH (19:1) to afford TN-20 (**1**). It contained a sulfur atom as indicated by combustion analysis. The UV spectrum was similar to that of (±)-corynoline (**2**). The MS of TN-20 (**1**) showed a very weak fragment peak at *m/e* 367 which corresponds to the M^+ of (±)-corynoline (**2**). ((±)-Corynoline (**2**) showed a molecular ion peak of high intensity at *m/e* 367.) The ^1H NMR spectrum of TN-20 (**1**) in DMSO-d_6 showed a C-Me and a N-Me peak, two methylenedioxy peaks, 4 aromatic proton signals and a peak exchangeable with D_2O (Table 1,a). The ^{13}C NMR spectrum in DMSO-d_6 contained 21 C atoms (Table 2). The multiplicities for each carbon obtained from off-resonance decoupling experiments corresponded to those for each carbon of (±)-corynoline (**2**) and showed 20 hydrogens attached to the carbon atoms.

The ^1H NMR and ^{13}C NMR spectra of TN-20 (**1**) showed an alternation in the signals when the probe temperature was raised to *ca* 100°. The ^1H NMR spectrum measured after treatment at *ca* 100° in DMSO-d_6 or DMSO-d_6–C_5D_5N (*ca* 3:1) for 10 min showed the other signals (B line) together with the signals (A line) observed at room temperature. The spectra recorded after treatment at 100° for 1–3 hr in each solution showed only the signals of the B line. These spectra were similar to those of the hydrochloride and the sulfate of (±)-corynoline (**2**) (Table 1, a and b). The ^{13}C NMR spectrum of TN-20 recorded after accumulation for *ca* 2 hr at 100° in DMSO-d_6–C_5D_5N (*ca* 3:1) differed from the room temperature spectrum (Table 2). This spectrum remained unchanged as the probe temperature was lowered to room temperature (B and C line in Table 2) and it was in agreement with that of the sulfate of (±)-corynoline (**2**) (Table 2). The ^1H NMR spectrum recorded after treatment at 100° in C_5D_5N for 10 min differed from the room temperature spectrum and comprised only the signals of the B line (Table 1, c). Heating of TN-20 at 100° in DMSO-d_6–C_5D_5N (*ca* 3:1), and purification, yielded (+)-corynoline (**2**), which had an identical IR spectrum to that of naturally occurring (+)-corynoline (**2**). On the basis of the spectral examinations and upon the result of solvolysis, TN-20 was assumed to be the 11-*O*-sulfate of (+)-corynoline (**2**).



1 R = SO_3H
2 R = H
3 R = Ac

The 11-*O*-sulfate of (±)-corynoline was prepared from (±)-corynoline (**2**) by reaction with pyridine–sulfur trioxide complex [5] in $CHCl_3$. Since the synthetic sample is sparingly soluble in most organic solvents, identification of the product by IR spectrum in a solution with the natural base could not be carried out, but it was identical with the natural alkaloid by ^1H NMR (in trifluoroacetic acid) (Table 1, d) and TLC R_f value. On the

* Part XXXIV in the series "Studies on the Alkaloids of Papaveraceous Plants". For Part XXXIII see Takao, N. and Iwasa, K. (1979) *Chem. Pharm. Bull. (Tokyo)*, in press.

Table 1. ^{1}H NMR spectral data for TN-20 (**1**) and corynoline (**2**) derivatives

		C(13)-Me	N-Me	C(12)-H_2	C(6)-H_2	C(14)-H	C(11)-H	OCH_2O	C(1)-H	C(4)-H	C(9)-H C(10)-H	NH or OH
[a in DMSO-d_6]												
TN-20 (**1**)	A*	1.23	2.9	3.22, *d-d* (19, 4) 3.64, *d-d* (19, 1)	4.50 4.73 ABq (15)	4.5	4.73	6.09 *br s* 6.11 *br s*	6.92	7.12	7.01 7.17 ABq (8.4)	8.39
	B‡	1.2	2.79	3.12, *d* (3)	4.52 4.56 ABq (16)	4.52	4.18	6.06, *m*	6.86	7.17	7.0 7.11 ABq (8)	
	C†	1.18	2.77	3.09, *br s*	4.53 ABq (16) overlapping	4.53	4.18	6.1, *m*	6.8	7.21	7.04 7.14 ABq (8)	9.67
(±)-Corynoline (**2**)	A*	1.06	2.19	2.95, *d* (2.8)	3.47 3.89 ABq (16)	over-lapping	3.81	6.01 *br s*	6.74	6.88	6.86 7.01 ABq (8.4)	7.16
(±)-Corynoline hydrochloride (2 HCl)	A*	1.16	2.82	3.11, *br s*	4.62 *br s*	4.79	4.19	6.09 *br s* 6.11 *br s*	6.9	7.37	7.04 7.14 ABq (8.5)	9.71
	B†	1.19	2.82	3.14, *br s*	4.62 *br s*	4.73	4.19	6.04 *br s* 6.08 *m*	6.83	7.33	6.99 7.11 ABq (8.5)	
(±)-Corynoline sulfate (2 H_2SO_4)	A*	1.22	2.89	3.16, *br s*	4.66 *br s*	over-lapping	4.24	6.14 *br s*	6.93	7.36	7.02 7.18 ABq (8.5)	
	B†	1.21	2.86	3.14, *br s*	4.63 *br s*	4.5	4.22	6.07 *br s*	6.84	7.29	7.0 7.11 ABq (8)	
[b in DMSO-d_6–C_5D_5N]												
TN-20 (**1**)	A*	1.48	3.0	3.27, *d-d* (18, 4.5) 3.53, *d-d* (18, 5)	4.31 4.46 ABq (16)	4.43	4.94 *t* (5)	6.07 *m* 6.13 *br s*	6.77	7.23	6.98 7.4 ABq (8.4)	
	B†	1.21	2.6	3.13, *d* (3)	4.11 4.36 ABq (16)	4.14 *d* (2)	4.08	6.04 *m*	6.79	7.1	6.93 7.09 ABq (8)	
	C‡	1.24	2.77	3.2, *d* (2.5)	4.39 4.56 ABq (15.5)	4.41	4.26	6.17 *m* 6.19 *br s*	6.8	7.3	7.04 7.14 ABq (8.5)	
(±)-Corynoline hydrochloride (2 HCl)	A*	1.23	2.81	3.22, *d* (3)	4.55 *br s*	4.55	4.27	6.16 *br s*	6.87	7.43	7.0 7.14 ABq (8.4)	
	B†	1.2	2.63	3.16, *d* (3)	4.26 4.34 ABq (16)	4.13 *d* (3)	4.22	6.06 *br s*	6.79	7.21	6.93 7.1 ABq (8.4)	
[c in C_5D_5N]												
TN-20 (**1**)	A*	1.58	3.07	3.39, *d-d* (18, 4.5) 3.9, *d-d* (18, 5)	4.51 4.56 ABq (16)	4.53	5.36 *t* (5)	5.64 *br s* 5.83 *br s*	6.53	7.38	6.84 7.52 ABq (8.4)	
	B†	1.2	2.29	3.17, *d-d* (18, 4.5) 3.2, *d-d* (18, 2.6)	3.66 4.14 ABq (15)	3.59	4.14	5.91 *m* 5.94 *br s*	6.71	6.92	6.87 7.07 ABq (8)	
	C‡	1.22	2.41	3.21, *d-d* (18, 4) 3.26, *d-d* (18, 2)	3.9 4.27 ABq (15)	3.86	4.22	6.0 *m* 6.07 *br s*	6.78	7.16	6.93 7.09 ABq (8)	
[d in CF_3CO_2H]												
TN-20 (**1**)	A*	1.49	3.11 *d* (5.5)	3.39, *d-d* (20, 3) 3.78, *d-d* (20, 1)	4.39, *d-d* (15.5, 10) 4.94, *d-d* (15.5, 2)	4.33 *d* (9)	5.33 *d* (3)	6.07 *br s*	6.87	6.97	7.02 7.18 ABq (8.5)	7.78
(±)-Corynoline acetate (**3**)	A*	1.53	3.14 *d* (5.5)	3.42, *d-d* (20, 4) 3.36, *d-d* (20, 1)	4.6, *d-d* (16, 10) 4.92, *d-d* (16, 2)	4.49 *d* (9)	5.69 *d* (4)	6.1 *br s*	6.84	7.01	7.03 7.11 ABq (8.5)	7.5

* Values recorded at a probe temperature of *ca* 35°.
† Values recorded after the spectrum changed completely at a probe temperature of *ca* 100°.
‡ Values recorded as the probe temperature was lowered from 100° to 35°.
Values in parentheses represent approximate coupling constants (*d* = doublet, *bs s* = broad singlet, ABq = AB quartet, *m* = multiplet).

basis of irradiation experiments, the proton signals of TN-20 were assigned as shown in Table 1, d. The observed couplings of H-6, H-14 and N-Me protons with the NH proton were in agreement with those obtained for (±)-corynoline acetate (**3**) in trifluoroacetic acid.

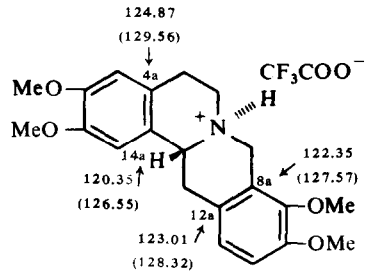
The 11-*O*-sulfate of (+)-corynoline was also prepared from (+)-corynoline (**2**) by treatment with pyridine–sulfur trioxide complex in $CHCl_3$ and had an identical IR spectrum to that of the natural alkaloid. TN-20 was therefore shown to be (+)-corynoline 11-*O*-sulfate.

The ^{1}H NMR and ^{13}C NMR spectra were examined in order to determine the structure for TN-20 in solution. The ^{1}H NMR spectrum of TN-20 in DMSO-d_6 showed the signals at lower field relative to those observed for (±)-corynoline (**2**). A similar low field shift was observed between the salt and free base of (±)-corynoline (Table 1, a). In the ^{13}C NMR spectrum in DMSO-d_6, the signals for C-1a, C-6a and C-10a were found at

Table 2. Carbon-13 spectral data for

		C-Me	C-12	C-Me	N-Me	C-6	C-14	C-11	OCH_2O		C-1
[in DMSO-d_6]											
TN-20 (**1**)	A*	23.14 (*q*)	31.3 (*t*)	40.09 (*s*)	41.63 (*q*)	51.57 (*t*)	67.65 (*d*)	78.86 (*d*)	101.47 (*t*)	101.7 (*t*)	108.52 (*d*)
(±)-Corynoline (**2**)	A*	23.34 (*q*)	36.32 (*t*)	40.37 (*s*)	42.44 (*q*)	52.79 (*t*)	68.42 (*d*)	74.79 (*d*)	100.81 (*t*)	101.03 (*t*)	107.02 (*d*)
[in DMSO-d_6–C_5D_5N]											
TN-20 (**1**)	A*	24.78	31.94	40.98	42.4	50.17	67.77	78.86	101.49	101.65	108.12
	B†	22.95	35.4	40.89	41.53	52.15	68.68	74.37	101.46‡	101.77‡	108.32‡
	C§	22.7	35.02	40.48	41.31	51.81	67.77	73.93	101.54	101.88	108.52
(±)-Corynoline sulfate (2 H_2SO_4)	A*	22.59	34.8	40.42	41.16	51.56	67.68	73.96	101.59	101.85	108.82

The indication in parentheses shows multiplicities obtained from ^{1}H off-resonance experiments.
* Values recorded at a probe temperature of *ca* 35°.
† Values recorded after the spectrum changed completely at a probe temperature of *ca* 100°.
‡ These values were read normally.
§ Values recorded as the probe temperature was lowered from 100° to 35°.



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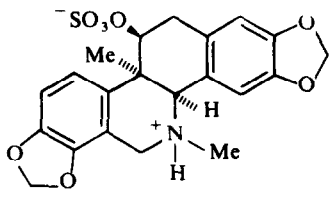

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higher fields than those in (±)-corynoline (Table 2). These high field shifts at C-1a (γ position for N atom), C-10a (γ position) and C-6a (β position) might be due to the effects of *N*-protonation on ^{13}C chemical shift as observed for C-4a (γ position for N atom), C-8a (β position), C-12a (γ position) and C-14a (β position) in the ^{13}C NMR spectrum (**4**) of the salt of tetrahydropalmatine in $CDCl_3$ and trifluoroacetic acid [5]. Therefore TN-20 appears to adopt an inner salt structure in DMSO (**5**).

Thus, the 11-*O*-sulfate of (+)-corynoline was isolated from *Corydalis incisa* Pers. The occurrence of naturally occurring sulfates of steroid hormones and lipids from animal tissues and metabolites is well known [7]. This is the first report to our knowledge of a sulfate of an alkaloid occurring in higher plants. The fact that optically active corynoline was isolated as a sulfate is of interest in connection with the metabolism of this alkaloid in the plant.

EXPERIMENTAL

Mps are uncorr. TLC and PLC were carried out on Si gel PF_{254}. ^1H NMR spectra were recorded at 90 MHz at probe temps. of *ca* 35°, and/or *ca* 50° and *ca* 100°. Samples were dissolved in DMSO-d_6 or C_5D_5N, or DMSO-d_6–C_5D_5N (*ca* 3:1) or CF_3CO_2H containing TMS as internal standard. The ^{13}C NMR spectra were measured at 22.6 MHz in 8 mm tubes at probe temps. of *ca* 35°, and/or *ca* 50° and *ca* 100°. Samples were dissolved in DMSO-d_6 or DMSO-d_6–C_5D_5N (*ca* 3:1) containing TMS as an internal standard at a concn of *ca* 0.2–0.5 mol/l. Conditions of the FT NMR measurements were: spectral width, 5000 Hz; pulse width, 25–30 μsec; acquisition time, 0.8 sec; number of data points, 8192.

Isolation and identification of TN-20 (**1**). The tertiary nonphenolic fraction (TN-A fraction), separated by the procedure previously described [2], was applied to a Si gel column and eluted successively with hexane–EtOAc (from 4:1 to 100% EtOAc), MeCOEt and MeCOEt satd with H_2O. Rechromatography of the fraction eluted with MeCOEt using Si gel with $CHCl_3$–MeOH (19:1) afforded TN-20, colorless prisms, mp 253–254° ($CHCl_3$–MeOH), $[\alpha]_D^{23}$ +67° (MeOH, *c* 0.19); IR ν_{max}^{nujol} cm^{-1}: 3100; UV λ_{max}^{MeOH} nm: 237 (ε 4.13) and 289 (ε 4.02); MS *m/e*: 367 ($M^+ - SO_3$, weak), 365 (weak), 363 (weak), 349, 334, and 318. (Found: C, 56.17; H, 4.73; N, 2.98; S, 7.15. Calc. for $C_{21}H_{21}NO_8S$: C, 56.37; H, 4.73; N, 3.13; S, 7.17%).

Conversion of TN-20 (**1**) *to* (+)-*corynoline* (**2**). TN-20 (100 mg) was heated in 0.5 ml DMSO-d_6–C_5D_5N (*ca* 3:1) at probe temp. for *ca* 2 hr. The soln was concd under red. pres. and the residue separated by PLC on Si gel using MeOH–Et_2O (1:1) followed by C_6H_6–Et_2O (3:7). This afforded an alkaloid which was recrystallized from MeOH–Et_2O to give (+)-corynoline (**2**), 57 mg, mp 180–181°, $[\alpha]_D^{23}$ +132° ($CHCl_3$, *c* 2.64) identical with naturally occurring (+)-corynoline by IR (KBr).

Preparation of 11-O-*sulfate of* (±)-*corynoline* (**1**). (±)-Corynoline (0.2 g) in $CHCl_3$ (5 ml) was shaken at room temp. with Py–sulfur trioxide (1 g) for 2.5 hr. To this soln, Py–sulfur trioxide (1 g) was added and the mixture shaken for 2 hr and then heated under reflux for 5 hr. After cooling, the white crystalline ppt. formed was filtered off, washed with hot MeOH repeatedly to remove unreacted Py–sulfur trioxide, and dried in a vaccum desiccator for several hr. These crystals, mp 268–272°, were sparingly soluble in MeOH, $CHCl_3$, *n*-BuOH, *sec*-BuOH, *n*-PrOH, and *iso*-PrOH; IR ν_{max}^{nujol} cm^{-1}: 3105; ^1H NMR (CF_3CO_2H): δ 1.5 (3H, *s*, C-Me), 3.11 (3H, *d*, J = 5.3 Hz, N-Me), 6.08 (4H, *br s*, OCH_2O × 2), 6.87 (1H, *s*, Ar-H), 6.97 (1H, *s*, Ar-H), 7.02, 7.17 (each 1H, *d*, J = 8.4 Hz, Ar-H); MS *m/e*: 367 (weak), 365 (weak) 363 (weak), 349, 334, 318. (Found: C, 56.65; H, 4.85; N, 2.19. Calc. for $C_{21}H_{21}NO_8S$: C, 56.37; H, 4.73; N, 3.13%).

Preparation of 11-O-*sulfate of* (+)-*corynoline* (**1**). (+)-Corynoline (45 mg) in $CHCl_3$ (4 ml) was shaken with Py–sulfur trioxide (1 g) for 3.5 hr at room temp. and heated under reflux for 1.5 hr. After addition of Py–sulfur trioxide (500 mg), the mixture was heated under reflux for 1.5 hr. The ppt. formed was filtered off and extracted with $CHCl_3$. From the $CHCl_3$ extract, the main product and unreacted (+)-corynoline were separated by PLC

TN-20 (**1**) and corynoline (**2**) derivatives

C-9	C-4	C-6a	C-10	C-1a	C-4a	C-10a	C-7	C-2, 3, and 8		
109.05 (*d*)	112.77 (*d*)	110.69 (*s*)	120.44 (*d*)	117.94 (*s*)	128.97 (*s*)	131.57 (*s*)	142.11 (*s*)	145.23 (*s*)	145.47 (*s*)	149.11 (*s*)
108.8 (*d*)	112.38 (*d*)	116.72 (*s*)	118.78 (*d*)	125.43 (*s*)	127.48 (*s*)	136.08 (*s*)	142.12 (*s*)	144.31 (*s*)	144.84 (*s*)	147.33 (*s*)
109.03	111.55	112.29	120.99	120.99	129.1	132.15	142.52	145.18	145.84	148.57
109.29‡	113.1‡	113.54‡	119.47‡	121.24‡	128.76‡	134.51‡	142.21‡	145.62‡	146.02‡	149.12‡
109.25	113.33	112.63	119.92	119.41	128.52	133.92	142.64	145.35	145.63	148.96
109.34	113.53	111.84	119.53	118.99	128.81	133.47	142.68	145.65	145.65	149.16

on Si gel (C_6H_6–Et_2O, 1:4). The main product was further purified by repeated PLC on Si gel ($CHCl_3$–MeOH, 7:3). The ppt. from the reaction mixture was dissolved in MeOH and then purified by PLC on Si gel ($CHCl_3$–MeOH, 9:1) to afford an alkaloid identical with the above main product. The alkaloid obtained, which was recrystallized from MeOH to give white crystals, mp 252–255°, was identical with natural TN-20 by IR (KBr) and MS.

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