Isolation and Structure Elucidation of the Alkaloids of *Delphinium glaucescens* Rybd.

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A comprehensive study of the basic components of *Delphinium glaucescens*, a toxic larkspur indigenous to the western United States, has led to the isolation of five new C_{19}-diterpenoid alkaloids and nine known alkaloids. The known alkaloids, listed in order of decreasing abundance, are lycoctonine (**16**), dictyocarpine (**2**), browniine (**9**), 14-dehydrobrowniine (**8**), methyllycaconitine (**12**), delcosine (**15**), dictyocarpinine (**7**), deltaline (**1**), and anthranoyllycoctonine (**17**). Dictyocarpinine had not been isolated previously as a natural product. The structures of four of the new alkaloids, namely, glaucenine (**3**), glaucerine (**4**), glaucephine (**5**), and glaucedine (**10**), were firmly established by synthesis. Alkaloids **3**, **4**, and **10** contain ester groups which were previously unknown in the C_{19}-diterpenoid alkaloids. The 2-methylbutyryl esters of **3** and **10** were determined to have the (*S*)-(+) configuration. The fifth new alkaloid, glaudelsine, was assigned structure **13** on the basis of its ^{13}C NMR spectrum and the proton NMR spectrum of its hydrolysis product.

Larkspur (*Delphinium* sp.) poisoning frequently affects cattle on ranges of the western United States, and annual death losses of 12% may occur.[1] *Delphinium glaucescens* Rybd. grows primarily on sagebrush slopes of Custer County, ID, and Madison County, MT, where cattle losses by death from poisoning by this plant have often been reported.[2] Relative toxicity of larkspur varies according to alkaloid content[3] and differences among species have been reported.[4-7] It may be that relative toxicity of a species can be better correlated with content of certain individual alkaloids than with total alkaloid content per se. Our investigations can be of fundamental value in this regard.

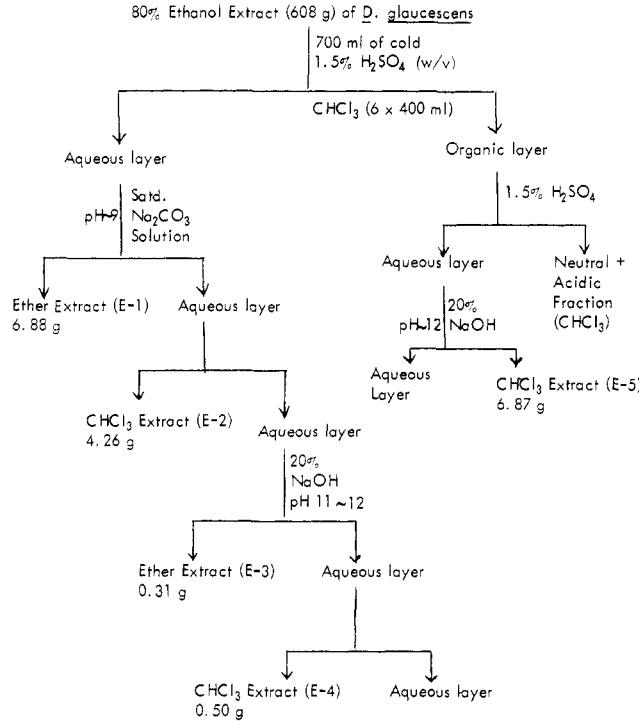
Delphinium glaucescens has been examined for alkaloid content,[5,6] and the toxicity of crude extracts has been investigated.[4] In 1910, Beath[5] reported the isolation of one white crystalline alkaloid exhibiting low toxicity. *D. glaucescens* was found to contain 0.25% to 0.60% of alkaloids depending upon the season collected. To our knowledge, a systematic investigation of the alkaloidal components of *D. glaucescens* has not previously been undertaken. This paper describes the isolation and structure elucidation of five new C_{19}-diterpenoid alkaloids as well as the isolation of nine known C_{19}-diterpenoid alkaloids.

Results and Discussion

Extraction of the aerial parts of *D. glaucescens* with 80% ethanol, followed by processing of the extract as shown in Scheme I, afforded a mixture of crude alkaloids in ca. 0.75% yield.

The ether extract at pH 9 (E-1) yielded the five known C_{19}-diterpenoid alkaloids: deltaline (**1**), dictyocarpine (**2**), 14-dehyrobrowniine (**8**), browniine (**9**), and methyllycaconitine (**12**) (see Charts I and II). In addition, dictyocarpinine (**7**), not previously isolated as a natural product, was obtained. Details of the isolation and identification of the components of E-1 are given in the Experimental Section.

Scheme I



Dictyocarpinine (**7**), the saponification product of **2**,[8] has not been isolated previously from natural sources. In order to clarify whether or not the dictyocarpinine isolated is an artifact, a solution of **2** in methanol was stirred in the presence of alumina (activity III). No detectable **7** was found after 24 h. Alumina was the only adsorbent used in the isolation process.

In addition to **2**, **8**, and **9** the chloroform extract at pH 9 (E-2) furnished delcosine (**15**) and a new alkaloid, glaudelsine (**13**). Furthermore, at least two other uncharac-

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(2) Personal communication by J. D. Olsen, to USDA Forest Service Office, Dubois, ID.
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(4) Olsen, J. D. *J. Range Manage.* **1977**, *30*, 237.
(5) Beath, O. A. *J. Am. Pharm. Assoc.* **1910**, *7*, 955.
(6) Beath, O. A. *Bull.—Wyo., Agric. Exp. Stn.* **1919**, *No. 120*, 55.
(7) Beath, O. A. *Bull.—Wyo., Agric. Exp. Stn.* **1925**, *No. 143*, 49.
(8) Narzullaev, A. S.; Yunusov, M. S.; Yunusov, S. Y. *Khim. Prir. Soedin.* **1972**, *8*, 498.

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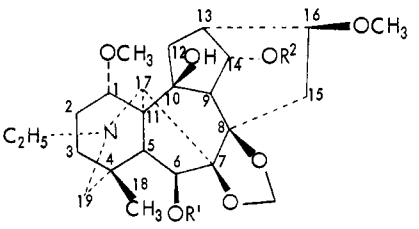
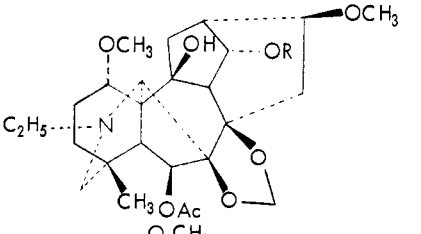
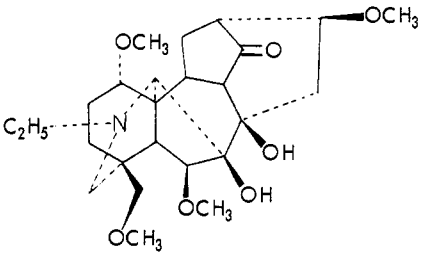
Chart I



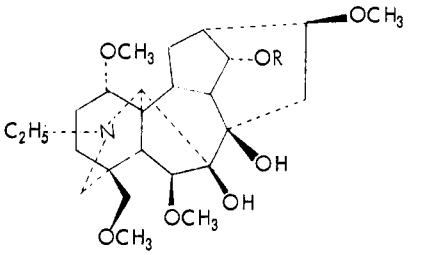
1 R' = Ac; R^2 = CH_3 Deltaline
2 R' = Ac; R^2 = H Dictyocarpine
6 R' = R^2 = Ac
7 R' = R^2 = H Dictyocarpinine



3 R = $-C(=O)-C(CH_3)(H)-CH_2-CH_3$ Glaucenine
4 R = $-C(=O)-CH(CH_3)_2$ Glaucerine
5 R = $-C(=O)-C_6H_5$ Glaucephine

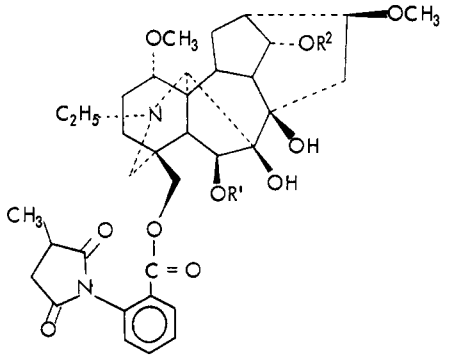


8 14-Dehydrobrowniine

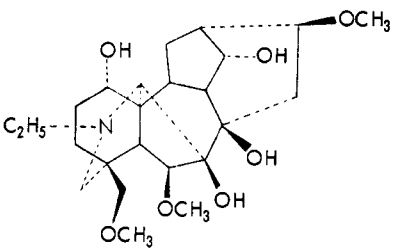


9 R = H Browniine
10 R = $-C(=O)-C(CH_3)(H)-CH_2CH_3$ Glaucedine
11 R = $-C(=O)-CH_3$

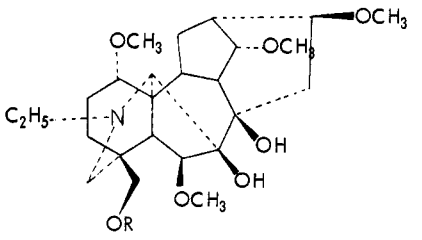
Chart II



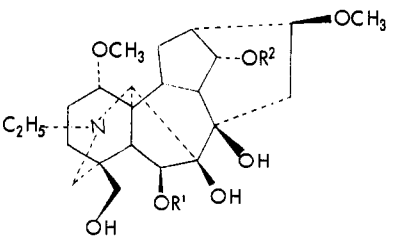
12 R' = R^2 = CH_3 Methyllycaconitine
13 R' = H; R^2 = CH_3 Glaudelsine
14 R' = CH_3; R^2 = H



15 Delcosine



16 R = H Lycoctonine
17 R = $-C(=O)-C_6H_4-NH_2$ Anthranoyllycoctonine



18 R' = H; R^2 = CH_3
19 R' = CH_3; R^2 = H Delectinine

Table I. Alkaloidal Components of *Delphinium glaucescens*

compd	approx amt isolated, mg	mp, °C	specific rotation,[b] deg
deltaline (1)	167	186.5-188	-30.0 (MeOH)
dictyocarpine (2)	1620	214.5-216.5	-14.7 ($CHCl_3$)
glaucenine (3)	105[a]	amorphous	-45.0 ($CHCl_3$)
glaucerine (4)	63[a]	amorphous	-48.5 ($CHCl_3$)
glaucephine (5)	26[a]	amorphous	-33.6 ($CHCl_3$)
dictyocarpinine (7)	183[a]	205-206.5	-5.0 (MeOH)
14-dehydrobrowniine (8)	630	172-174	+31.4 ($CHCl_3$)
browniine (9)	1580	amorphous	+38.7 (EtOH)
browniine perchlorate		211.5-213	+29.8 (EtOH)
glaucedine (10)	133[a]	117-120	+39.1 (MeOH)
methyllycaconitine (12)	400	amorphous	+48.1 (EtOH)
glaudelsine (13)	23[a]	amorphous	+36.1 ($CHCl_3$)
delcosine (15)	356[a]	203.5-202.5	+57.4 ($CHCl_3$)
lycoctonine (16)	3340	95-97.5	+52.8 (EtOH)
anthranoyllycoctonine (17)	52[a]	amorphous	+50.1 (EtOH)

[a] Amount of highly purified material isolated. [b] Solvent in parentheses.

terized alkaloids containing anthranilic acid esters were also isolated.

Delcosine was isolated by column chromatography on alumina by using 2% methanol/acetone as the eluent. Diacetone alcohol was formed during the chromatography. We observed that the dimerization of acetone was facilitated in the prescence of alumina alone. About 390 mg of diacetone alcohol was isolated after stirring 100 mL of acetone in the presence of 10 g of alumina (activity III) for 5 h.

Column chromatography of fraction E-5 on silica gel furnished compounds **8**, **9**, lycoctonine (**16**), and anthranoyllycoctonine (**17**), as well as the novel alkaloidal esters glaucenine (**3**), glaucerine (**4**), glaucephine (**5**), and glaucedine (**10**). [Dr. O. E. Edwards has kindly informed us that X-ray analysis of two new degradation products of lycoctonine (**16**) has shown that the configuration of the C(1)-methoxyl is α, in contrast to that reported in the original X-ray analysis. The configuration of the C(1)-methoxyl in lycoctonine and related alkaloids discussed in this paper is therefore shown in the α configuration.]

Fractions E-3 and E-4 have not been examined to date. They were given lower priority since they represented a small percentage of the total alkaloids and had many components with no single component predominating.

All of the characterized alkaloids isolated from fractions E-1, E-2, and E-5 are listed in Table I. In order to give an indication of relative abundances, the approximate amounts of each of the alkaloids isolated are given. In general, all fractions containing a given alkaloid were not highly purified, and the approximations represent an estimate of total crude alkaloid. In several cases the amount of pure alkaloid isolated is given and is so denoted. All known crystalline alkaloids were characterized by their melting points and specific rotations (Table I). Browniine (**9**) was characterized by its perchlorate. The ^1H NMR spectra of all compounds were recorded (Table II). The ^1H NMR spectra of alkaloids **1**, **2**, **7**–**9**, **12**, and **15**–**17** are consistent with those previously reported.[9-11] The chemical shifts reported in the Soviet literature[9,10] are referenced to hexamethyldisiloxane as an internal standard. Consequently, the chemical shifts for compounds **1**, **2**, and **7** appearing in Table II are 0.02 - 0.17 ppm higher than the corresponding values appearing in the literature.[9,10]

The ^{13}C NMR spectra of alkaloids **8**, **9**, **12**, and **15**–**17** are in accord with those previously reported.[12,13] The ^{13}C NMR spectra of the methylenedioxy-group-containing alkaloids **1**, **2**, and **7** had not been reported previously. We have discussed the **13C** NMR spectra of these compounds as well as those of the derivative **6** in an earlier paper.[14] The structures of the new alkaloids **3**, **4**, **5**, **10**, and **13** were determined largely on the basis of their ^{13}C NMR spectra. The chemical shifts of these compounds are reported in Table III. The chemical shifts for 14-acetyldictyocarpine (**6**), 14-acetylbrowniine (**11**), and lycoctonine (**16**) are included for comparison purposes.

Characterization of Glaudelsine (13). Glaudelsine was isolated from fraction E-2 as a white amorphous solid (23 mg). The material appeared as a single homogeneous highly UV-active spot upon examination by TLC. The proton NMR showed the presence of three methoxy groups and an anthranilic acid moiety. The ^{13}C NMR of glaudelsine clearly indicated that it possessed the basic structure of methyllycaconitine (**12**), with the replacement of one methoxy group by a hydroxy group (Table III). The indicated structure ($C_{36}H_{48}N_2O_{10}$) was confirmed by the mass spectrum (M^+, m/e 668). The ^{13}C NMR spectrum of glaudelsine exhibited chemical shifts for methoxy groups at 56.0, 56.5, and 58.3 ppm. The higher field chemical shifts at 56.0 and 56.5 ppm are diagnostic for methoxy groups at C(1) and (16).[15] Consequently, only structures **13** and **14** need be considered for glaudelsine. Of these, **13** is the most probable structure. A signal at 75.3 ppm generally shows the presence of a hydroxy group at C(14).[15] Such a signal is absent in the spectrum of glaudelsine. Furthermore, an examination of the ^1H NMR spectra of **1** and **2** (Table II) clearly establishes the chemical shift for the C-14 methoxy group in **1** at 3.49 ppm, in excellent agreement with the observed chemical shift at 3.52 ppm in the spectrum of glaudelsine.

In order to establish unambigously the structure of glaudelsine, a 13-mg sample was subjected to alkaline hydrolysis. The isolated amino alcohol (6.7 mg) had the following: mp 72–83 °C; $[\alpha]^{17}_D$ + 32° (c 0.21, $CHCl_3$). Its proton NMR spectrum shows methyl groups at 1.04 (triplet), 3.29, 3.41, and 3.48 ppm (singlets).

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(10) Narzullaev, A. S.; Yunusov, M. S.; Yunusov, S. Y. *Khim. Prir. Soedin.*
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(15) Pelletier, S. W.; Sawhney, R. S. *Heterocycles* **1978**, *9*, 463.

Table II. Proton NMR Spectral Data of C_{19}-Diterpenoid Alkaloids[a]

	C(18)-CH_3	NCH_2CH_3	$OC(O)CH_3$	OCH_3	methylenedioxy	C(6)H	C(14)H	other CH_3
deltaline (1)	0.90	1.07	2.07	3.29, 3.36, 3.49	4.97, 5.01	5.53 (d, J = 2 Hz)	4.12 (d, J = 6 Hz)	
dictyocarpine (2)	0.88	1.08	2.12	3.30, 3.39	5.00, 5.05	5.55 (d, J = 1.5 Hz)	4.70 (dd, J = 6, 6 Hz)	
glaucenine (3)	0.89	1.06	2.05	3.32 (2)	4.89, 4.97	5.46 (d, J = 1.5 Hz)	5.28 (dd, J = 6, 6 Hz)	0.97 (t, J = 7.5 Hz), 1.12 (d, J = 7 Hz)
glaucerine (4)	0.90	1.07	2.07	3.32, 3.35	4.91, 4.98	5.45 (s)	5.32 (dd, J = 6, 5 Hz)	1.17 (d, J = 7 Hz)
glaucephine (5)	0.88	1.06	2.05	3.31, 3.33	4.89, 4.95	5.48	5.48	
14-acetyldictyocarpine (6)	0.90	1.08	2.08 (2)	3.32, 3.35	4.97, 5.03	5.53 (br s)	5.36 (dd, J = 5.5, 5.5 Hz)	
dictyocarpinine (7)	0.93	1.06		3.26, 3.38	5.12, 5.24	4.28 (d, J = 1 Hz)	4.72 (dd, J = 6, 5 Hz)	
14-dehydrobrowniine (8)		1.10		3.33, 3.35 (2), 3.45		3.93		
browniine (9)		1.05		3.27, 3.32, 3.38, 3.44		3.90 (d, J = 1 Hz)	4.05 (dd, J = 7, 4 Hz)	
glaucedine (10)		1.03		3.28, 3.33 (2), 3.43		3.88 (d, J = 3 Hz)	4.82 (dd, J = 5, 5 Hz)	0.90 (t, J = 7.5 Hz), 1.15 (d, J = 7 Hz)
methyllycaconitine (12)		1.08		3.28, 3.38, 3.42, 3.45		4.15 (d, J = 1 Hz)	3.98 (dd, J = 4, 1 Hz)	1.47 (d, J = 6 Hz)
glaudelsine (13)		1.08		3.29, 3.41, 3.52		4.16 (d, J = 0.5 Hz)	3.92	1.48 (d, J = 6.5 Hz)
glaudelsimine (18)		1.04		3.29, 3.41, 3.48		4.27	3.85	

[a] The solvent is chloroform-*d* with Me_4Si as an internal standard.

Of the two possible hydrolysis products, only one, delectinine (**19**), is known.[16] Delectinine has a reported melting point of 167–169 °C and an $[\alpha]_D$ +42° (*c* 0.67, $CHCl_3$). The aforementioned physical data strongly suggest that the hydrolysis product must have structure **18**.

Establishment of Structures of Glaucenine (3), Glaucerine (4), Glaucephine (5), and Glaucedine (10). Fraction E-5 was chromatographed on a silica gel column. Elution with 3% methanol/dichloromethane afforded fractions 33–43 which contained glaucenine (**3**), glaucerine (**4**), glaucephine (**5**), and 14-dehydrobrowniine (**8**). The separation of the dictyocarpine esters **3–5** was quite tedious and was best accomplished by multiple (five or six) developments on alumina PLC plates using 10–12% acetone/hexane. Further elution of the column with 4% methanol/dichloromethane afforded fractions 65–69, which upon preparative layer chromatography (alumina, ethyl acetate) afforded the new alkaloid glaucedine (**10**) and anthranoyllycoctonine (**17**). Similarly, browniine (**9**) and **10** were isolated from fractions 70–75 (eluted with 5% methanol/dichloromethane) and dictyocarpinine (**7**) and **10** from fractions 76–81 (eluted with 5% and 6% methanol/dichloromethane).

The structures of compounds **3–5** and **10** were deduced from their ^{13}C NMR spectra and confirmed by synthesis of authentic samples. In each case, the ^{13}C NMR spectrum of the synthetic material matched that of the isolated alkaloid.

With racemic 2-methylbutyryl chloride, synthetic 14-(2-methylbutyryl)browniine (**10**) was obtained as a white solid: mp 98.5–100.5 °C (acetone/hexane); $[\alpha]^{27}_D$ +15° (*c* 0.25, MeOH). These physical constants differed markedly from those of the isolated material, as expected, since the naturally occurring material should be the ester of only one enantiomer of 2-methylbutyric acid. For the determination of the absolute configuration of the **10** isolated, a 26-mg sample was hydrolyzed. The isolated acidic fraction (1.48 mg) exhibited $[\alpha]^{26}_D$ +3° (*c* 0.11, MeOH), indicating that the 2-methylbutyric acid has the *S* configuration.[17] However, the maximum reported specific rotation of (*S*)-2-methylbutyric acid is +19.8° (neat).[18] Since the measured rotation was a small percentage of the reported value and since only small quantities of **10** (and **3**) were available for hydrolysis, the synthesis of **3** and **10** with optically pure (*S*)-(+)-2-methylbutyric acid was deemed necessary to firmly establish the absolute configuration of the alkaloids. To that end, racemic 2-methylbutyric acid was treated with (+)-α-methylbenzylamine in accordance with the literature procedure.[19] The salt collected was repeatedly recrystallized until a constant specific rotation was reached. The crystalline salt isolated after 22 crystallizations showed the following: mp 99–100 °C; $[\alpha]^{22}_D$ +16.0° (*c* 10.6, CH_2Cl_2). A portion of this material was decomposed to yield (*S*)-2-methylbutyric acid, $[\alpha]^{22}_D$ +19.3° (*c* 10.4, CH_2Cl_2). Using a correction factor based upon previous readings, this value corresponds to +19.9° for neat acid.

It has been reported that conversion of (*S*)-(+)-2-methylbutyric acid to its acid chloride may result in 2–3% racemization.[20] In order to preclude racemization in the

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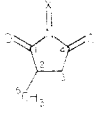
Table III. Carbon-13 Chemical Shifts and Assignments for New Alkaloids Isolated from *Delphinium glaucescens*[a]

atom	compd 3	4	5	6	10	11	12	13
C(1)	79.1	79.0	79.0	19.0	84.3	84.2	83.9	84.9
C(2)	26.9	26.9	26.9	27.0	26.2	26.2	26.0	25.3
C(3)	37.3	37.3	36.9	37.3	32.4	32.4	32.0	32.2
C(4)	33.7	33.7	33.8	33.7	37.1	38.1	37.6	37.0
C(5)	50.3	50.2	50.2	50.4	43.2	42.6	43.2	45.8
C(6)	77.3	77.3	77.4	77.3	90.5	90.3	90.8	90.3
C(7)	91.6	91.6	91.7	91.7	88.4	88.3	88.5	89.2
C(8)	83.2	83.2	83.2	83.3	77.4	77.1	77.4	76.3
C(9)	50.1	49.9	50.1	49.9	51.1	51.2	50.3	50.2
C(10)	81.3	81.2	81.2	81.3	38.1	38.1	38.0	37.9
C(11)	55.8	55.7	55.7	55.8	49.6	49.5	49.0	48.3
C(12)	36.6	36.5	36.6	36.6	28.3	28.2	28.7	27.6
C(13)	38.9	38.8	38.7	38.9	45.7	45.7	46.1	46.1
C(14)	74.1	74.3	74.3	74.7	75.6	76.0	83.9	84.9
C(15)	34.9	34.8	35.1	35.0	33.8	33.7	33.6	33.1
C(16)	81.2	81.2	81.2	81.3	82.3	82.4	82.5	81.7
C(17)	63.8	63.9	64.1	63.9	64.8	64.8	64.5	65.0
C(18)	25.3	25.6	25.6	25.6	78.1	78.0	69.5	69.5
C(19)	56.9	56.9	56.9	56.9	52.8	52.7	52.3	52.4
$NC(CH_3)H_2$	50.4	50.4	50.4	50.4	48.9	48.8	50.9	51.2
$NC(CH_3)H_2$	13.8	13.9	13.9	13.9	14.2	14.2	14.0	14.3
OCH_2O	93.7	93.7	93.9	93.8				
C(1)'[b]	55.4	55.4	55.5	55.4	55.8	55.8	55.7	56.1
C(6)'					57.4	57.3	57.8	
C(14)'							58.1	58.3
C(16)'	55.8	55.9	55.9	56.1	55.8	56.2	56.3	56.5
C(18)'					59.0	59.0		
$C(6)OC(CH_3)=O$	170.0	170.1	170.2	170.0				
$C(6)OC(CH_3)=O$	21.6	21.6	21.6	21.7				
C(14)OC(CH)=O	176.9				176.9	171.9		
C(14)OC(CH)=O	41.3	34.2			41.3			
(CH_3)	16.2	18.9			16.2	21.5		
(CH_2)	26.3				26.2			
(CH_3)	11.4				11.6			
			166.9				164.1	164.2
 1			130.7				127.1	127.0
2			129.9				133.1	133.1
3			128.3				129.4	129.5
4			132.7				133.6	133.7
5			128.3				131.0	131.0
6			129.9				130.0	130.1
 1							179.8	179.8
2							37.0	37.0
3							35.3	35.3
4							175.8	175.9
5							16.4	16.5

[a] Chemical shifts in parts per million downfield from Me_4Si; the solvent is deuteriochloroform. [b] Values given for primed carbons refer to chemical shifts for methoxyls.

esterification of dictyocarpine (**2**) and browniine (**9**) to form **3** and **10**, respectively, we sought a method employing mild conditions. To this end, the method of Hassner and Alexanian was investigated.[21] Treatment of **2** with optically pure (*S*)-(+)-2-methylbutyric acid, *N,N'*-dicyclohexylcarbodiimide (DCC), and *p*-(dimethylamino)pyridine (DAP) in dichloromethane at reflux temperature for 48 h afforded 14-(2-methylbutyryl)dictyocarpine in a yield of 76%. The product showed $[\alpha]^{21}_D$ −45.2° (*c* 4.3, $CHCl_3$), in excellent agreement with the measurement for isolated glaucenine (−45.0°). In a similar fashion, **9** was converted to **10** by using optically pure (*S*)-(+)-2-methylbutyric acid. Upon recrystallization, compound **10** exhibited a melting point of 113–118 °C and $[\alpha]^{17}_D$ +39.9° (MeOH), in agreement with the corresponding measurements for glaucedine isolated from the plant [mp 117–120 °C, $[\alpha]^{20}_D$ +39.1° (MeOH)].

Thus the absolute configurations of the esters **3** and **10** have been firmly established. Compounds **3**, **4**, and **10** thus represent the first known diterpenoid alkaloids containing either isobutyryl or 2-methylbutyryl ester functionalities. However, there are several examples of the occurrence of these two ester groups in other types of alkaloids. For example, the tropine alkaloids butropine (isobutyryltropine) and valtropine [(*R*)-2-methylbutyryltropine] have been isolated from *Duboiseia leichhardtii*,[22] and a number of veratrum alkaloids containing esters of (*S*)-(+)-2-methylbutyric acid have been reported.[23]

Experimental Section

General Methods. Proton nuclear magnetic resonance (^1H NMR) spectra were recorded of chloroform-*d* solutions on Varian T-60 and EM-390 spectrometers; chemical shifts are reported in parts per million (δ) from internal tetramethylsilane. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet, q, quartet; m, multiplet; br, broad. Carbon-13 NMR experiments

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were performed at 15.03 MHz in the Fourier mode with a JEOL FX-60 spectrometer in conjunction with a JEC-980 computer. The spectra were obtained at 30 °C in chloroform-*d* (which provided the lock signal) solutions. Carbon-13 chemical shifts are referenced in parts per million from internal Me_4Si. Samples were contained in precision-ground 5-mm-o.d. tubes. On the average, a 5-μs pulse, corresponding to an approximate tilt angle of 45°, was employed. For the spectral width of 4000 Hz, the delay between pulses was 2.5 s. Acquisition times averaged 2–48 h over 8K data points for concentrations of the order of 0.10–0.50 M. Mass spectra were recorded on a Finnegan Quadrapole 4023 mass spectrometer and a Du Pont 21-490 mass spectrometer at an ionizing voltage of 70 eV and an ion current of 290 μA. Infrared (IR) spectra were obtained on a Perkin-Elmer Model 297 spectrophotometer and were calibrated with the 1601- and 1028-cm^{-1} bands of polystyrene. Melting points were determined on a Thomas-Kofler hot stage equipped with a microscope and polarizer and are corrected. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter. Microanalyses were performed by Atlantic Microlab, Inc.

Analytical thin-layer chromatography (TLC) was carried out on Merck aluminum oxide PF-254 (Type E) or a 1:1 mixture of Merck silica gel H and silica gel HF-254 + 366. Preparative TLC was performed on Merck aluminum oxide 150, PF-254 + 366 (Type T) or a 1:1 mixture of silica gel H and silica gel HF-254 + 366. Either Merck silica Gel 60 (70–230 mesh ASTM) or aluminum oxide (activity stage III) prepared from Merck neutral aluminum oxide (90 active, activity stage I, 70–230 mesh ASTM) was used for column chromatography. The compositions of all solvent mixtures used in column and thin-layer chromatography were determined on a volume/volume basis.

Isolation of Crude Alkaloids from *Delphinium glaucescens*. The aerial parts of *D. glaucescens* were collected in July 1977, during the early-bud to late-flower stage, northeast of Humphrey, ID. The dried and ground plant material (6.4 kg) was placed into four glass percolators and extracted with 80% ethanol (96 L total). The solvent was removed in vacuo at 21–29 °C to yield 1550 g of tarlike residue. A portion of the extract (608 g) was treated as shown in Scheme I. A total of 18.8 g of crude alkaloids was extracted, representing a 0.75% yield from the whole plant.

Isolation of the Components of E-1. The ether extract at pH 9 (E-1) was placed upon a column containing 500 g of activity III alumina and eluted successively with hexane, 10% ether/hexane, 20% ether/hexane, 20% ethyl acetate/hexane, and 50% ethyl acetate/hexane (125–250-mL fractions) to afford ca. 0.08 g of nonalkaloidal material. Continued elution with ethyl acetate gave the following results: fractions 11–14, 0.30 g, one major and two minor components; fraction 15, 0.11 g, one alkaloidal component; fractions 16–21, 1.46 g, three components, one probably identical with fraction 15; fractions 22–39, 3.30 g, two components. Subsequent elution with 2%, 5%, 10%, and 20% methanol/ethyl acetate and finally pure methanol yielded eight to ten minor alkaloids.

Fractions 11–14. Deltaline (1). Preparative layer chromatography of fractions 11–14 on alumina with 10% ethyl acetate/ether as the developing solvent and extraction of the most polar band with 20% ethanol/chloroform afforded 167 mg of deltaline (1),[24-26] also known as eldeline.[27] Recrystallization from ether gave 101 mg of material: mp 186.5–188 °C (lit.[25] mp 180–181 °C, lit.[27] mp 182–184 °C); $[\alpha]^{26}_D$ -30.0° (*c* 1.1, MeOH) [lit.[26] $[\alpha]^{23}_D$ -28.5° (MeOH)]. The proton[8,9] and ^{13}C NMR spectra[14] of deltaline are consistent with the assigned structure.

Fraction 15. 14-Dehydrobrowniine (8). The ^{13}C NMR spectrum of the noncrystalline material isolated from this fraction indicated that it was crude 14-dehydrobrowniine.

Fractions 16–21. Isolation of 14-Dehydrobrowniine (8), Browniine (9), and Methyllycaconitine (12). Fractions 16–21 were combined (1.46 g) and chromatographed on a column containing 125 g of silica gel. Elution with 500 mL of chloroform and 950 mL of 1% methanol/chloroform gave no alkaloids. Continued elution with 1% methanol/chloroform (375 mL) and 2% methanol/chloroform (200 mL) afforded 0.39 g of 14-dehydrobrowniine (8) which crystallized upon being allowed to stand; mp 163–166 °C (lit.[28] mp 163–164 °C). Recrystallization of a 0.30-g portion from ether–ethyl acetate gave crystals melting at 160.5–163 °C (first crop, 78 mg) and at 172–174 °C (second crop, different crystalline form, 118 mg). The lower melting crystals exhibited $[\alpha]^{23}_D$ + 31.3° (*c* 1.07, 95% EtOH) and $[\alpha]^{17}_D$ +30.4° (*c* 0.96, $CHCl_3$), and the higher melting crystals showed $[\alpha]^{17}_D$ +31.4° (*c* 1.15, $CHCl_3$), at considerable variance with the reported[28] value, $[\alpha]^{27}_D$ +19°. The ^{13}C NMR spectra of the two crystalline forms were identical and in accord with the literature[12] with one exception. The chemical shifts for C(2) and C(12) had been reported as 25.5 and 29.7 ppm, respectively. In actuality, the chemical shifts for C(2) and C(12) are 25.5 and 25.3 ppm. The previously reported peak at 29.7 ppm can be attributed to hydrocarbon impurities.

Continued elution with 2% methanol/chloroform (275 mL) gave 0.15 g of amorphous material. This material was assigned the structure of browniine (9)[29] on the basis of its ^1H and ^{13}C NMR spectra.[12,13] Reaction of a sample of 9 with 60% perchloric acid in methanol (1:10) afforded browniine perchlorate as a white solid which crystallized from methanol/ethyl acetate: mp 211.5–213 °C (lit.[29] mp 212 °C); $[\alpha]^{19}_D$ +29.8° (*c* 0.98, EtOH) [lit.[11] $[\alpha]_D$ +25.4° (EtOH)]. A 26.7-mg sample of browniine perchlorate was decomposed with 5% aqueous NaOH in methanol to yield 21.8 mg (99%) of browniine, $[\alpha]^{27}_D$ +38.7° (*c* 1.09, EtOH).

Further elution gave 0.53 g of a mixture of 9 and a UV-active material and then 0.23 g of the UV-active material as an amorphous white solid, mp 100–110 °C. Repeated attempts at crystallization of the latter were unsuccessful, resulting in some decomposition. The material was identified as methyllycaconitine (12)[30] on the basis of its ^1H and ^{13}C NMR spectra.[13] The crude 12 was further purified by preparative TLC on silcia gel by using 10% methanol/chloroform as the developing solvent. Extraction of the UV-active band yielded 88 mg of 12 as a noncrystalline white solid: mp 139–142 °C (lit.[30] mp 128 °C); $[\alpha]^{21}_D$ +48.1° (*c* 2.68, EtOH) [lit.[30] $[\alpha]^{22}_D$ +49.1° (*c* 2.0, EtOH)].

Elution with 5% methanol/chloroform gave 40.8 mg of a three-component mixture and 26.5 mg of a single alkaloid. These minor constituents have not yet been investigated.

Fractions 22–37. Isolation of Dictyocarpine (2), Browniine (9), and Dictyocarpinine (7). Recrystallization of fractions 22–37 (3.21 g) from ether plus a trace of dichloromethane and then from toluene afforded 189 mg of a compound (mp 211.5–214 °C) which has been assigned the structure of dictyocarpine (2, lit.[8] mp 210–212 °C). The ^{13}C and proton[9] NMR spectra are consistent with the assigned structure.

The material recovered from the mother liquors (2.94 g) was chromatographed on an alumina column. Elution with 20% ether/chloroform gave 0.73 g of crude browniine (9) and 0.20 g of a mixture of 9 and dictyocarpine (2). Crude 2 (1.07 g) was eluted with 20% ether/chloroform and chloroform. Recrystallization from hexane/acetone furnished an analytical sample: mp 214.5–216.5 °C; $[\alpha]^{22}_D$ -12.8° (*c* 1.04, MeOH); $[\alpha]^{24}_D$ -14.7° (*c* 0.97, $CHCl_3$) [lit.[16] $[\alpha]_D$ -14° (*c* 0.76, $CHCl_3$).

Further elution with 1% and 5% methanol/chloroform gave 0.31 g of six minor constituents. The major fraction (0.21 g) was chromatographed on alumina plates with 6% methanol/chloroform. Extraction of the most polar band afforded 74 mg of dictyocarpinine (7, mp 193.5–197 °C), whose proton NMR was in accordance with that reported in the literature.[9] Recrystallization from hexane–acetone increased the melting point to 199.5–202 °C (lit.[8] mp 204–205 °C); $[\alpha]^{21}_D$ - 4.6° (*c* 0.80, MeOH).

Isolation of Components of E-2. The chloroform extract of crude alkaloids at pH 9 (E-2, 4.26 g) was chromatographed on a column containing 300 g of silica gel. Elution with 1.75 L of dichloromethane of 4 L of 2% methanol/dichloromethane af-

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Table IV. Column Chromatography of Fraction E-2

fractions	eluent used (v/v)	weight of material, g	description of material isolated
20-23	2% MeOH/CH_2Cl_2	0.14	crude 14-dehydrobrowniine (8)
24-29	2.5% MeOH/CH_2Cl_2		
30-35	3% MeOH/CH_2Cl_2	0.03	mixture of 8 and at least three others
36-42	4% MeOH/CH_2Cl_2	0.26	primarily two components, one UV active
43-48	4% MeOH/CH_2Cl_2	0.46	above constituent (not UV active) plus dictyocarpine (2)
49-51	4% MeOH/CH_2Cl_2	0.06	previous two components plus dark green material
52-56	4% MeOH/CH_2Cl_2	0.13	at least four components, including dark green material and browniine (9)
57-75	4% MeOH/CH_2Cl_2	0.45	crude 9 and two minor constituents, and UV active
76-82	5% MeOH/CH_2Cl_2		
83-93	5% MeOH/CH_2Cl_2	0.10	two components (new)
94-96	5% MeOH/CH_2Cl_2	0.14	two UV-active highly polar alkaloidal components
97-112	6% MeOH/CH_2Cl_2		
113-115	8% MeOH/CH_2Cl_2	0.07	
116-120	10% MeOH/CH_2Cl_2		
121-125	20% MeOH/CH_2Cl_2	0.22	mixture of three additional alkaloids
126-127	30% MeOH/CH_2Cl_2	0.19	tan solid (one UV active spot on TLC)
128-129	30% MeOH/CH_2Cl_2	0.26	mixture of previous and subsequent isolated materials
130-131	30% MeOH/CH_2Cl_2	0.13	tan solid (one UV active spot on TLC)
132-133	30% MeOH/CH_2Cl_2	0.11	as above, plus a trace of lower R_f material
134-136	40% MeOH/CH_2Cl_2		
137-138	50% MeOH/CH_2Cl_2	0.06	complex mixture
139	50% MeOH/CH_2Cl_2	0.03	oil, one extremely polar alkaloidal component
140	pure MeOH		

forded no alkaloid fraction. Continued elution (250–500-mL fractions) gave the results shown in Table IV.

The identities of previously isolated alkaloids (14-dehydrobrowniine, dictyocarpine, and browniine) were confirmed by proton and carbon-13 NMR spectral analysis.

Fractions 36–42. Glaudelsine (13) and Delcosine (15). Fractions 36–42 (0.26 g) were chromatographed on a column containing 40 g of alumina (activity III). Elution with 1% methanol/acetone and 1.5% methanol/acetone gave 0.03 g of dictyocarpine (2). Continued elution with 1.5% methanol/acetone gave a mixture of UV-active material plus diacetone alcohol. The mixture was dissolved in 50 mL of cold 1.5% sulfuric acid, extracted with dichloromethane (2 × 50 mL), basified with saturated sodium carbonate solution to pH 9, and extracted with dichloromethane. Removal of solvent gave 26.0 mg of material which was washed three times with hexane. The resulting amorphous solid (23.5 mg, mp 80–110 °C) was assigned structure **13** on the basis of its ^1H and ^{13}C NMR spectra: $[\alpha]^{21}_D$ +36.1° (*c* 0.97, $CHCl_3$); IR ($CHCl_3$) 3470 (br), 2930, 1714 (sh), 1453, 1390, 1258, 1086 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.08 (t, 3, J = 7.5 Hz, NCH_2CH_3), 1.48 (d, 3, J = 6.5 Hz, CH_3), 3.29 (s,3, OCH_3), 3.41 (s, 3, OCH_3), 3.52 (s, 3, OCH_3), 3.92 (m, 1, C(14) H), 4.16 (d, 1, J = 0.5 Hz, C(6) H), 7.18–8.25 ppm (m, 4, aromatic); mass spectrum, m/z (relative intensity) 668 (1), 637 (1), 279 (2), 167 (29), 149 (100), 113 (7), 83 (33), 71 (38).

A 19-mg sample of **13** was treated with 6% perchloric acid in methanol to give 22 mg of salt. Attempted crystallization from methanol/ether gave an amorphous solid, mp 203–213 °C.

Continued elution with 2–10% methanol/acetone gave a mixture of **15** and diacetone alcohol. The material was treated with 25 mL of cold 1.5% sulfuric acid. The aqueous solution was extracted with dichloromethane (2 × 25 mL). Then the aqueous solution was basified with saturated sodium carbonate solution and extracted with dichloromethane (3 × 25 mL). Removal of solvent gave 106 mg of **15**, mp 187.5–190 °C. Recrystallization from methanol plus a trace of ether and from acetone provided 71 mg of **15**, mp 198.5–201 °C (lit.[31] mp 203–204 °C).

Fractions 43–48. Dictyocarpine (2) and Delcosine (15). Fractions 43–48 (457 mg) were recrystallized from acetone plus a trace of hexane yielding 76 mg of **2**. The material recovered from the mother liquor was partitioned between an aqueous sodium carbonate solution (pH 8) and dichloromethane. Removal of solvent gave 66 mg of **2**. The pH of the aqueous solution was increased to ~11 (NaOH) and extracted with dichloromethane. Removal of solvent gave 250 mg of **15**, mp 190.5–194 °C. Recrystallization from acetone gave a sample of delcosine: mp 203.5–205.5 °C; $[\alpha]^{25}_D$ +57.4° (*c* 1.23, $CHCl_3$) [lit.[31] $[\alpha]^{25}_D$ +56.8° (*c* 2.01, $CHCl_3$)].

Isolation of Components of E-5. Fraction E-5 was chromatographed on a column containing 475 g of silica gel. Elution with 1 L of dichloromethane, 2 L of 1% methanol/dichloromethane, 1 L of 1.5% methanol/dichloromethane, and 1.6 L of 2% methanol/dichloromethane afforded no alkaloid. Continued elution (250-mL fractions) gave the results shown in Table V.

The number of components in the fractions described above was determined by the number of spots observed upon TLC on alumina with ethyl acetate as the developing solvent for fractions 22–101 and 8% methanol/acetone for fractions 102–162. A component described as "new" had not appeared in a previous fraction.

Fractions 33–43. Glaucenine (3), Glaucerine (4), and Glaucephine (5). Fractions 38–43 were placed upon two 20 cm × 40 cm × 2.5 mm alumina plates and developed with 20% ethyl acetate/ether. Extraction of the higher R_f band with 20% ethanol/dichloromethane and 20% methanol/dichloromethane gave 41.2 mg of glaucenine (**3**). Extraction of the lower R_f band gave 28.7 mg of glaucerine (**4**). Extraction of the two overlapping bands yielded 43.8 mg of a mixture of **3**–**5**. The mixture was chromatographed on a 20 cm × 20 cm × 2 mm alumina plate with 10% acetone/hexane (four developments) to give 16.2 mg of **3**, 19.5 mg of **4**, and 5.7 mg of glaucephine (**5**) upon extraction with 20% ethanol/dichloromethane and 20% methanol/dichloromethane.

Fractions 33–37 (245.8 mg) were placed upon three 40 cm × 20 cm × 3 mm alumina plates, and the plates were developed with 12% acetone/hexane five times to give 42.1 mg of **3** plus traces of **4**, 28.0 mg of **4** plus traces of **3** and **5**, 23.9 mg of **5** plus traces of **4**, and 90.5 mg of 14-dehydrobrowniine (**8**). Rechromatography on alumina with 10% acetone/hexane (six developments) gave 20.3 mg of pure **3**, 14.9 mg of pure **4**, and 20.3 mg of pure **5**, as well as 27.7 mg of **3** plus a trace of impurities and 7.7 mg of a mixture of **4** and **5**.

A pure sample of glaucenine (**3**) exhibited the following physical properties: $[\alpha]^{26}_D$ −45.0° (*c* 0.58, $CHCl_3$); IR ($CHCl_3$) 3460 (w), 2960, 2930, 2875, 1730 (sh), 1460, 1367, 1252, 1155, 1128, 1090, 1083 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.89 (s, 3, CH_3), 0.97 (t, 3, J = 7.5

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Table V. Column Chromatography of Fraction E-5

fractions	eluent used (v/v)	weight (mg)	description of material isolated
22-27	2% MeOH/CH_2Cl_2	73	crude 14-dehydrobrowniine (**8**)
29-32	3% MeOH/CH_2Cl_2	69	14-dehydrobrowniine and one minor component
33-37	3% MeOH/CH_2Cl_2	246	mixture of **8** and three new components
38-43	3% MeOH/CH_2Cl_2	159	mixture of above three new components
44-46	3% MeOH/CH_2Cl_2	37	above two components plus two more
47-50	3% MeOH/CH_2Cl_2	33	six components, two new
51-54	3% MeOH/CH_2Cl_2	103	five components, at least one new
55-60	4% MeOH/CH_2Cl_2		
61-64	4% MeOH/CH_2Cl_2	126	green oil: four components, two new
65-69	4% MeOH/CH_2Cl_2	205	five components, two new
70-72	5% MeOH/CH_2Cl_2	96	four of above five components
73-75	5% MeOH/CH_2Cl_2	83	
76-81	6% MeOH/CH_2Cl_2	261	two of above four components
82-87	6% MeOH/CH_2Cl_2	122	eight-component mixture, three new
88-90	6% MeOH/CH_2Cl_2	52	three of above eight components
91-93	10% MeOH/CH_2Cl_2		
94-96	10% MeOH/CH_2Cl_2	58	two components, one new
97-101	10% MeOH/CH_2Cl_2	500	two components, one new; new component identified as lycoctonine (**15**)
102-106	10% MeOH/CH_2Cl_2	1924	crude lycoctonine
107-115	15% MeOH/CH_2Cl_2		
116-127	20% MeOH/CH_2Cl_2		
128-137	25% MeOH/CH_2Cl_2	316	lycoctonine plus small amount of very polar material
138-142	40% MeOH/CH_2Cl_2		
143-145	40% MeOH/CH_2Cl_2	135	lycoctonine plus traces of at least two highly polar constituents
146-149	50% MeOH/CH_2Cl_2		
150-152	50% MeOH/CH_2Cl_2	147	lycoctonine plus trace of highly polar material
153-157	MeOH		

Hz, CH_3CH_2), 1.06 (t, 3, J = 7 Hz, CH_3CH_2N), 1.12 (d, 3, J = 7 Hz, CH_3), 2.05 (s, 3, $OCOCH_3$), 3.32 (s, 6, 2 OCH_3), 4.89 (s, 1, OCHO), 4.97 (s, 1, OCHO), 5.32 (dd, 1, J = 6, 6 Hz, C(14) H), 5.46 (br s, 1, C(6) H); mass spectrum, m/z (relative intensity) 577 (0.5), 563 (0.5), 547 (27), 546 (87), 518 (6), 166 (15), 150 (16), 122 (28), 85 (100), 71 (97).

A portion of amorphous **3** was converted to its perchlorate by treatment with 6% perchloric acid in methanol at 0 °C. Recrystallization of the isolated salt from methanol/hexane gave an analytical sample, mp 227.5–232.5 °C.

Anal. Calcd for $C_{31}H_{48}NO_{13}Cl$: C, 54.90; H, 7.13; N, 2.07; Cl, 5.23. Found: C, 54.82; H, 7.13; N, 2.06; Cl, 5.30.

A pure sample of glaucerine (**4**) exhibited the following properties: $[\alpha]^{28}_D$ -48.5° (c 1.5, $CHCl_3$), $[\alpha]^{27}_D$ -27.5° (c 1.13, EtOH); IR ($CHCl_3$) 3460 (w), 2955, 2930, 2875, 1737, 1733, 1729, 1727, 1467, 1458, 1367, 1245 (br), 1159, 1128, 1088 cm^{-1}; NMR ($CDCl_3$) δ 0.90 (s, 3, CH_3), 1.07 (t, s, J = 7 Hz, CH_3CH_2N), 1.17 (d, 6, J = 7 Hz, isopropyl CH_3), 2.07 (s, 3, $OCOCH_3$), 3.32 (s, 3, OCH_3), 3.35 (s, 3, OCH_3), 4.91 (s, 1, OCHO), 4.98 (s, 1, OCHO), 5.32 (dd, 1, J = 6, 5 Hz, C(14) H), 5.45 (s, 1, C(6) H); mass spectrum, m/z (relative intensity) 563 (2), 546 (2), 532 (62), 531 (17), 504 (7), 166 (7), 150 (8), 122 (16), 110 (9), 105 (8), 98 (10), 91 (8), 84 (13), 71 (100).

Anal. Calcd for $C_{30}H_{45}NO_9$: C, 63.92; H, 8.05; N, 2.49. Found: C, 63.68; H, 8.09; N, 2.42.

Chromatographically pure glaucephine (**5**) showed the following: $[\alpha]^{28}_D$ -33.6° (c 0.76, $CHCl_3$); IR ($CHCl_3$) 3470 (br, w), 2960, 2930, 2875, 1737, 1733, 1718, 1452, 1367, 1279, 1126, 1090, 1081 cm^{-1}; NMR ($CDCl_3$) δ 0.88 (s, 3, CH_3), 1.06 (t, 3, J = 7 Hz, CH_3CH_2N), 2.05 (s, 3, $OCOCH_3$), 3.31 (s, 3, OCH_3), 3.33 (s, 3, OCH_3), 4.89 (s, 1, OCHO), 4.95 (s, 1, OCHO), 5.48 (m, 2, C(6) and C(14) H), 7.38–7.65 (m, 3, para and meta aromatic H), 8.08–8.25 (m, 2, ortho aromatic H); mass spectrum, m/z (relative intensity) 566 (20, $M^+ - OCH_3$), 538 (1), 532 (1), 122 (33), 105 (100), 77 (86).

Anal. Calcd for $C_{33}H_{43}NO_9$: C, 66.31; H, 7.25; N, 2.34. Found: C, 66.09; H, 7.30; N, 2.31.

Fractions 70–75. Glaucedine (10). Fractions 70–72 (96 mg) and 73–75 (89 mg) were chromatographed on 40 cm × 20 cm × 2 mm alumina plates with ethyl acetate as the developing solvent. The most polar component (40.5 mg) of fractions 70–72 was positively identified as browniine (**9**). Fractions 73–75 also contained **9** (21.4 mg) plus a small amount (13.5 mg) of unidentified material of slightly higher R_f which has thus far not been completely separated from **9**. Extraction of the highest R_f band common to all fractions yielded 61 mg of crude glaucedine (**10**) as a white solid, mp 116–117.5 °C. Recrystallization from acetone/hexane gave an analytical sample: mp 117–120 °C; $[\alpha]^{27}_D$ +36.4° (c 0.81, MeOH); IR ($CHCl_3$) 3460 (br), 2930, 2875, 2825, 1721, 1383, 1157, 1135, 1090 cm^{-1}; NMR ($CDCl_3$) δ 0.90 (t, 3, J = Hz, CH_3CH_2), 1.03 (t, 3, J = 7.5 Hz, CH_3CH_2N), 1.15 (d, 3, J = 7 Hz, CH_3), 3.28 (s, 3, OCH_3), 3.33 (s, 6, 2 OCH_3), 3.43 (s, 3, OCH_3), 3.88 (d, 1, J = 3 Hz, C(6) H), 4.82 (dd, 1, J = 5.5 Hz, C(14) H); mass spectrum, (m/z, relative intensity) 551 (6), 536 (26), 520 (100), 506 (24), 490 (13), 488 (8), 85 (6), 71 (91), 57 (31).

Anal. Calcd for $C_{30}H_{49}NO_8$: C, 65.31; H, 8.95; N, 2.54. Found: C, 65.15; H, 8.99; N, 2.53.

Fractions 65–69. Anthranoyllycoctonine (17). The material isolated from fractions 65–69 was chromatographed on alumina plates with ethyl acetate as the developing solvent. Each of five bands was extracted with 20% ethanol/dichloromethane and then 15% methanol/dichloromethane. The highest R_f band yielded 26.2 mg of **10**. The fourth band from the top afforded 52.4 mg of amorphous white solid. The proton NMR spectrum of this material was identical with that of anthranoyllcoctonine (**17**). The isolated anthranoyllcoctonine exhibited $[\alpha]^{24}_D$ +50.2° (c 1.1, EtOH) [lit.[11] $[\alpha]^{24}_D$ +46.3° (c 0.55, EtOH), lit[32] $[\alpha]^{27}_D$ +51.2° (c 0.52, EtOH)]. In all, 172 mg of material was isolated from fractions 65–69.

Fractions 76–81. Glaucedine (10) and Dictyocarpinine (7). The material isolated from fractions 76–81 was chromatographed on alumina plates with ethyl acetate as the developing solvent. The first four bands were extracted with 15% ethanol/dichloromethane and 15% methanol/dichloromethane and afforded (in order of decreasing R_f) 46.2 mg of **10** and two other components 2.6, 5.6, and 47.7 mg). The constituents of the latter three bands have not to date been characterized. Recrystallization of crude **10** from acetone/hexane (twice) provided material with a melting point of 117–120 °C and $[\alpha]^{20}_D$ +39.1° (c 0.34, MeOH). Extraction of the most polar band with 15% methanol/dichloromethane gave 109 mg of crystalline white solid identified as dicytocarpinine (**7**). Recrystallization from acetone–hexane furnished material with a melting point of 200.5–205 °C and $[\alpha]^{26}_D$ -5.0° (c 1.1, MeOH).

Fractions 102–157. Lycoctonine (16). Analytical TLC revealed that fractions 102–157 contained essentially one component with trace amounts of impurities. This material was identified

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as lycoctonine (16) upon examination of its ^{13}C NMR spectrum. Recrystallization of fractions 105 and 107–115 from 70% ethanol furnished 569 mg of 16 colorless crystals: mp 95–97.5 °C (lit.[32] mp 96–97 °C); $[\alpha]^{24}_D$ +52.8° (c 1.5, EtOH) [lit.[30] $[\alpha]^{20}_D$ +53.2° (c 2, EtOH)].

Alkaline Hydrolysis of Glaudelsine (13). Glaudelsine perchlorate (12.9 mg, 0.19 mmol) was dissolved in 5 mL of methanol, and 1 mL of 5% aqueous sodium hydroxide was added. The mixture was allowed to react at room temperature for 24 h. The solvent was removed in vacuo, and the residue was partitioned between water (20 mL) and dichloromethane (3 × 20 mL). The combined organic layers were washed with brine (25 mL) and dried (K_2CO_3). Removal of solvent afforded 6.7 mg (76%) of 18. Attempted crystallization from acetone/hexane afforded an amorphous solid: mp 72–83 °C; $[\alpha]^{17}_D$ +32° (c 0.21, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.04 (t, 3, J = 7 Hz, CH_3CH_2N), 3.29 (s, 3, OCH_3), 3.41 (s, 3, OCH_3), 3.48 (s, 3, OCH_3), 3.85 (s, 1, C(14) H), 4.27 (s, 1, C(6) H); mass spectrum, m/z (relative intensity) 453 (5), 438 (35), 422 (100), 420 (36), 408 (6), 390 (8), 71 (54), 58 (82), 45 (37), 43 (37), 41 (45).

Synthesis of 14-Isobutyryldictyocarpine (4). A solution of 26.9 mg (0.054 mmol) of dictyocarpine, 12.0 mg (0.10 mmol) of p-(dimethylamino)pyridine (DAP), and 0.05 mL of triethylamine in 2 mL of dichloromethane was treated with 2 drops (excess) of isobutyryl chloride. After 18 h, the reaction was incomplete, and an additional 0.10 mL of triethylamine and 3 drops of acid chloride were added. Within a few minutes, the mixture turned dark orange. After 24 h, the solution was diluted with 10 mL of dichloromethane and washed with 10-mL portions of 5% sodium bicarbonate solution, water, saturated ammonium chloride solution, and brine and then dried (K_2CO_3). Removal of solvent gave 41 mg of brown-orange residue which was chromatographed on a column containing 15 g of alumina (activity III). Elution with ethyl acetate gave 29.2 mg (95%) of 4 as a colorless oil, $[\alpha]^{27}_D$ −29.2° (c 1.5, EtOH). The R_f value (Al_2O_3, EtOAc) and proton and ^{13}C NMR spectra of this material were identical with those of the isolated alkaloid, glaucerine, assigned structure 4. The isolated glaucerine had $[\alpha]^{27}_D$ −27.5° (c 1.13, EtOH).

Synthesis of 14-(2-Methylbutyryl)dictyocarpine (3). 2-Methylbutyryl chloride was prepared from the corresponding acid. Dictyocarpine (2; 40.6 mg, 0.082 mmol) was treated with 2-methylbutyryl chloride (0.08 mL, 0.7 mmol) and DAP (12 mg, 0.098 mmol) in 2 mL of pyridine for 48 h under nitrogen. The dark yellow solution was partitioned between water (25 mL) and dichloromethane (3 × 25 mL). The organic solution was washed with 25-mL portions of 5% sodium bicarbonate solution, saturated ammonium chloride solution, and brine and dried (Na_2SO_4). The solvent was removed in vacuo, and toluene was added to the residue and evaporated twice in order to remove residual pyridine. The crude product then obtained (135 mg) was chromatographed on a column containing 15 g of alumina with ethyl acetate as eluent to give 40.5 mg (85%) of 3 as a colorless oil. The R_f and ^1H and ^{13}C NMR spectra of this material were identical with those of the alkaloid, glaucenine, isolated from the plant.

Synthesis of 14-Benzoyldictyocarpine (5). A mixture of 43 mg (0.087 mmol) of 2, 0.10 mL (0.86 mmol) of benzoyl chloride, 12.0 mg (0.098 mmol) of DAP, and 2.0 mL of pyridine was stirred at room temperature under nitrogen for 18 h. Following the workup used for 4, 215 mg of crude product was isolated. This material was chromatographed on a column containing 15 g of alumina with ethyl acetate as eluent to furnish 30.0 mg (58%) of crude 5. This material was chromatographed on an alumina plate with ethyl acetate as the developing solvent. The UV-active band was extracted with 20% ethanol/dichloromethane to furnish 19.5 mg (37%) of pure 5 as a foam, $[\alpha]^{28}_D$ −32.3° (c 0.94, $CHCl_3$). Glaucephine isolated from the plant showed $[\alpha]^{28}_D$ −33.6° (c 0.76, $CHCl_3$).

Synthesis of 14-(2-Methylbutyryl)browniine (10). A mixture of 40.0 mg (0.086 mmol) of browniine, 0.08 mL (0.7 mmol) of 2-methylbutyryl chloride, 12.0 mg of DAP, and 2.0 mL of pyridine was stirred under nitrogen at room temperature for 64 h. Following the usual workup, 38.0 mg (81%) of crude 10 was isolated. The ^{13}C NMR spectrum of this material was identical with that of the alkaloid isolated. Recrystallization from acetone/hexane afforded material with a melting point of 98.5–100.5 °C and $[\alpha]^{27}_D$ +15° (c 0.25, MeOH).

Preparation of 14-Acetyldictyocarpine (6). A solution of dictyocarpine (103 mg, 0.21 mmol), DAP (26.7 mg, 0.22 mmol), and acetic anhydride (0.20 mL) in 5 mL of dry dichloromethane was stirred under nitrogen at room temperature (22 °C) for 9 h. The solution was diluted to 25 mL with dichloromethane and washed with 20-mL portions of 5% aqueous sodium bicarbonate solution, saturated ammonium chloride solution, and brine. Removal of solvent after drying (Na_2SO_4) gave 98.7 mg (88%) of 6 as a colorless oil. Trituration with hexane provided 6 as an amorphous white solid: mp 64–69.5° C; $[\alpha]^{28}_D$ −46.6° (c 1.0, $CHCl_3$); IR ($CHCl_3$) 3500 (w), 2960, 2950, 2930, 2890, 2885, 1735, 1366, 1249, 1089 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.90 (s, 3, CH_3), 1.08 (t, 3, J = 7 Hz, CH_3CH_2N), 2.08 (s, 6, 2 $OC(O)CH_3$), 3.32 (s, 3, OCH_3), 3.35 (s, 3, OCH_3), 4.97 (s, 1, OCHO), 5.03 (s, 1, OCHO), 5.36 (dd, 1, J = 5.5, 5.5 Hz, C(14) H), 5.53 (s, 1, C(6) H); mass spectrum, m/z (relative intensity) 535 (1), 520 (1), 505 (21), 504 (72), 476 (7), 446 (3), 71 (17), 58 (18), 43 (100).

A sample of 6 was treated with 5% perchloric acid in methanol at 0 °C. The isolated product was triturated with ether to furnish an analytical sample of perchlorate salt, mp 129–132.5 °C.

Anal. Calcd for $C_{28}H_{42}NO_{13}Cl$: C, 52.87; H, 6.66; Cl, 5.57. Found: C, 52.96; H, 6.71; Cl, 5.50.

Resolution of 2-Methylbutyric Acid. Isolation of Optically Pure (S)-(+)-2-Methylbutyric Acid.[19] To a solution of 66.5 g (0.65 mmol) of acid in 325 mL of petroleum ether/ether (1:1) was added a solution of 51.8 mL (48.7 g, 0.40 mmol) of (+)-α-methylbenzylamine in 325 mL of petroleum ether/ether (1:1). Crystallization was allowed to take place at −20 °C for 2 days. Collection of the crystals afforded 40.8 g of salt. Subsequent recrystallizations were performed from a minimum volume of ether/petroleum ether (3:1 to 6:1). In each case, the crystallization was allowed to begin at room temperature, and after approximately 6 h it was continued at 5 °C overnight.

Starting with the fourth crystallization and for approximately every other crystallization thereafter, the salt isolated from the mother liquor was decomposed. Typically, 30 mL of 10% sodium hydroxide solution or saturated sodium carbonate solution was added to the salt, and the aqueous layer was extracted with dichloromethane (3 × 25 mL) to recover (+)-α-methylbenzylamine. The aqueous layer was then acidified with concentrated HCl and extracted with dichloromethane (3 × 25 mL) to give 2-methylbutyric acid. The acid thus recovered from the eighth mother liquor exhibited $[\alpha]^{23}_D$ +6.28° (neat) or $[\alpha]^{22}_D$ +6.10° (c 10.5, CH_2Cl_2). After 22 crystallizations, the specific rotation of the acid retrieved from the salt isolated from the mother liquor reached a constant value. The values for the last three recrystallizations were as follows: $[\alpha]^{23}_D$ +18.4° (c 7.2, CH_2Cl_2), $[\alpha]^{19}_D$ +18.5° (c 4.0, CH_2Cl_2), $[\alpha]^{21}_D$ +18.5° (c 3.9, CH_2Cl_2). The crystalline amine salt isolated after 22 crystallizations amounted to 2.52; mp 99–100 °C. The material exhibited $[\alpha]^{22}_D$ +16.0° (c 10.6, CH_2Cl_2). The 23rd crystallization gave crystals with $[\alpha]^{23}_D$ +15.8° (c 10.2, CH_2Cl_2). The salt isolated from the mother liquor was combined with the crystalline material, and a 1.97-g portion was decomposed to yield 877 mg of 2-methylbutyric acid, $[\alpha]^{22}_D$ +19.3° (c 10.4, CH_2Cl_2). Using a correction factor based upon previous readings, this value corresponds to +19.9° for neat acid. The maximum reported value[18] is $[\alpha]^{25}_D$ +19.8°.

Reaction of Dictyocarpine (2) with Optically Pure (S)-(+)-2-Methylbutyric Acid.[21] A solution of 98.0 mg (0.20 mmol) of 2, 115 mg (1.13 mmol) of (S)-(+)-2-methylbutyric acid $[\alpha]^{22}_D$ +19.3° (CH_2Cl_2)), 233 mg (1.13 mmol) of N,N'-dicyclohexylcarbodiimide (DCC), and 34.5 mg (0.28 mmol) of DAP in 10 mL of dichloromethane was heated at reflux for 48 h. The white precipitate was collected, and the filtrate was treated with 25 mL of cold 1.5% sulfuric acid. The aqueous layer was extracted with dichloromethane (3 × 25 mL) and then basified with saturated sodium carbonate solution. The aqueous layer was extracted with dichloromethane (3 × 25 mL). The combined organic layers were washed with 25 mL of saturated ammonium chloride solution and 25 mL of brine and dried (Na_2SO_4). Removal of the solvent afforded 87 mg (76%) of 3 which exhibited $[\alpha]^{21}_D$ −45.2° (c, 4.3, $CHCl_3$), in excellent agreement with the measurement for a sample of glaucenine (−45.0°) isolated from the plant.

Reaction of Browniine (9) with Optically Pure (S)-(+)-2-Methylbutyric Acid.[21] A solution o 87.1 mg (0.186 mmol)

of browniine, 107 mg (1.05 mmol) of (*S*)-(+)-2-methylbutyric acid ($[\alpha]^{22}_D$ +19.3° (CH_2Cl_2)), 217 mg (1.05 mmol) of DCC, and 32.1 mg (0.26 mmol) of DAP in 10 mL of dichloromethane was heated at reflux for 20 h. The usual workup procedure furnished 74 mg of crude product which was placed upon a column containing 15 g of alumina (activity III). The column was eluted with ethyl acetate to yield 62.2 mg (61%) of 14-(2-methylbutyryl)browniine (**10**), mp 110.5–111.5 °C. Recrystallization from hexane/acetone twice afforded **10** with a melting point of 113–118 °C and $[\alpha]^{17}_D$ +39.9° (*c*, 0.72, MeOH), in excellent agreement with the corresponding measurements for a sample of glaucedine isolated from the plant.

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Registry No. 1, 6836-11-9; 2, 59989-92-3; 3, 78018-27-6; 3 perchlorate, 78018-28-7; 4, 78018-29-8; 5, 78018-30-1; 6, 75659-26-6; 6 perchlorate, 78018-31-2; 7, 50657-27-7; 8, 4829-56-5; 9, 5140-42-1; 9 perchlorate, 5005-20-9; 10, 78039-66-4; 11, 65601-04-9; 12, 21019-30-7; 13, 78018-32-3; 13 perchlorate, 78018-33-4; 15, 545-56-2; 16, 26000-17-9; 17, 22413-78-1; 18, 78018-34-5; 19, 58480-82-3; isobutyryl chloride, 79-30-1; 2-methylbutyryl chloride, 5856-79-1; benzoyl chloride, 98-88-4; (+)-α-methylbenzylamine, 3886-69-9; (*S*)-(+)-2-methylbutyric acid, 1730-91-2.

Synthesis and Chemistry of a Stabilized Dehydrosecodine Model System[1]

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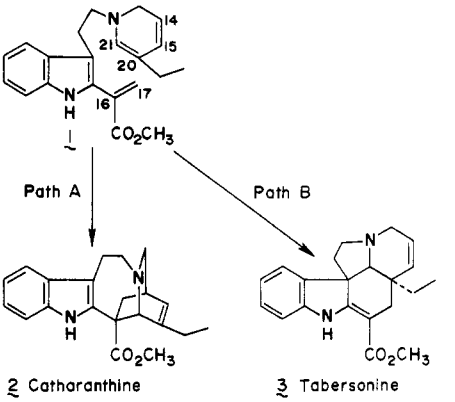
A stabilized dehydrosecodine analogue bearing carbomethoxy groups in the 3- and 5-positions of the dihydropyridine moiety has been prepared and its chemistry studied. Two novel procedures have been developed for this synthesis: (1) the Lewis acid assisted cleavage of an activated indole ether with trimethylsilyl cyanide to form a cyano alcohol and (2) the oxidation of 2-(α-substituted ethyl)indoles with *tert*-butyl hypochlorite to form the corresponding 2-vinylindole derivatives. Thermal decomposition of the dehydrosecodine analogue does not yield the desired intramolecular Diels–Alder adducts but instead seems to proceed by an intramolecular hydride transfer from the 1,2-dihydropyridine moiety to the vinylindole group.

Experimental evidence in support of the Thomas–Wenkert monoterpenoid hypothesis[2] for the biosynthesis of the indole alkaloids led Scott to propose a modified mechanistic scheme for the biosynthesis of the *Aspidosperma* and *Iboga* alkaloids (Scheme I).[3] The pivotal intermediate in this scheme, 14,21-dehydrosecodine (**1**),[4] might undergo an intramolecular Diels–Alder reaction in either of two ways (Scheme I): in path A, the dihydropyridine reacts as a diene leading to the formation of catharanthine (**2**); in path B, the dihydropyridine serves as the dienophile leading to the formation of tabersonine (**3**). Unfortunately, dehydrosecodine **1** or even dihydropyridines related to **1** have not been isolated or synthesized because of their propensity toward oxidation, dimerization, and polymerization.

Büchi and co-workers[5] used an *inter*molecular Diels–Alder reaction (path A type) between 1-benzyl-3-cyano-1,6-dihydropyridine and methyl vinyl ketone in their syntheses of ibogamine and ibogaine. Ziegler and Spitzner[6] used an *inter*molecular reaction (path B type) between methyl α-(*N*-methylindol-2-yl)acrylate and 1-benzyl-3-ethyl-1,4,5,6-tetrahydropyridine in a biogenetically patterned synthesis of (±)-minovine. Kuehne and co-workers[7] subsequently reported the related *intra*molecular Diels–Alder reaction (path B type) of the biogenetically postulated secodine isomer (14,15-dihydro **1**) to give vincadifformine (14,15-dihydro **3**). Most recently, Fowler and co-workers[8] have elegantly demonstrated that the *inter*molecular Diels–Alder reaction between ethyl α-(*N*-methylindol-2-yl)acrylate and *N*-methyl-1,2-dihydropyridine does indeed proceed along both pathways A and B as depicted in Scheme I to give *Aspidosperma*- and *Iboga*-type products in a 2.3:1 ratio. Several other laboratories[9] have recently reported progress toward the syn-

Scheme I



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