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TETRALUDINS D TO N, ELEVEN NEW MELAMPOLIDES FROM *TETRAGONOTHECA LUDOVICIANA*

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Key Word Index—*Tetragonotheca ludoviciana*; Compositae; Heliantheae; melampolides; sesquiterpene lactones.

Abstract—Eleven new melampolides, tetraludins D–N, were isolated from aerial parts of *Tetragonotheca ludoviciana*. Tetraludins D and E, F and G, J and K, and L and M represented epimeric pairs which differed only at one chiral centre of the five-carbon ester side chain at C-8. In benzene-d_6, the ^{1}H NMR spectra of the diastereomeric pairs of melampolides exhibited well-separated signals in particular for the absorptions due to the ester side chains that differed in chirality.

INTRODUCTION

In continuation of our biochemical systematic study within the family Compositae we have further investigated *Tetragonotheca ludoviciana* of the subtribe Heliantheneae. In addition to the previously described tetraludins A–C [1], eleven new melampolides, tetraludins D–N, were isolated from the aerial parts of the plant. All new compounds had the same medium ring skeleton and they differed only in the type of ester side chains at C-8 and C-9. A number of compounds existed as non-separable diastereomeric mixtures which were successfully analysed by ^{1}H NMR spectroscopy in benzene-d_6. The structures of the new compounds were established by ^{1}H NMR spectroscopy including extensive spin-decoupling experiments as well as by MS and chemical transformations.

RESULTS AND DISCUSSION

The two diastereomeric mixtures, tetraludin D (**4**)–E (**5**), ($C_{26}H_{34}O_{10}$) and F (**6**)–G (**7**), ($C_{25}H_{32}O_{10}$) exhibited very distinct ^{1}H NMR absorptions that were nearly identical with those of the medium ring portion of tetraludin A (**1**) [1] and differed only in the signals due to the ester side chains at C-8 and C-9.

Tetraludin D (**4**), $C_{26}H_{34}O_{10}$, mp 139–140°, was obtained after repeated chromatography as a pure compound which exhibited ^{1}H NMR signals characteristic of the medium ring as summarized in Table 2. In $CDCl_3$, the signals due to H-5 and H-6 near 5 ppm represented a second order pattern which was clearly resolved into a broadened doublet at δ 4.46(H-5, J = 10 Hz) and a triplet at 4.95 (H-6) when run in C_6D_6. Further ^{1}H NMR signals of **4** were characteristic for the 2-methylbutyrate (B_1) [triplet at 0.82, doublet at 1.04 (J = 7.0 Hz)] and 2-methyl-2-hydroxy-3-ketobutyrate (E_1) with ^{1}H NMR methyl singlets at 1.50 and 2.14 [1]. Further evidence for the presence of the above two ester functions in **4** was provided by diagnostic MS peaks (Table 4).

Tetraludin E (**5**), $C_{26}H_{34}O_{10}$, mp 132–133° exhibited ^{1}H NMR signals nearly identical with **4** when run in $CDCl_3$. However, in C_6D_6 the ^{1}H NMR absorptions due to the ester side chains *B* and *E* showed distinct differences suggesting that **4** and **5** represent isomers either by the difference in attachments (*B* at C-8 and *E* at C-9 and vice versa) or due to a difference in chirality at C-2′ of *B* and/or C-2′ of *E*. Periodate oxidation of a mixture of **4** and **5** (Scheme 1) provided the pyruvate **15** which upon selective hydrolysis with $NaHCO_3$ in ether gave the alcohol **21** in which the C-8 proton absorption had shifted upfield by about 1 ppm. These conversions not only showed that in **4** and **5** the ester side chain *B* is attached to the respective C-9 positions and *E* to C-8, but also that the difference between **4** and **5** is due to the opposite chirality at C-2′ of *E* since both **4** and **5** formed one pyruvate (**15**).

Tetraludin F (**6**) and G (**7**) represented a mixture which could not be completely separated from **4** and **5**. The ^{1}H NMR spectrum together with the diagnostic MS peaks (Table 4) indicated that **6** and **7** differed from **4** and **5** by the attachment of an isobutyrate moiety (A_1) to C-9 in **6** and **7**. Conversion of a mixture of **4**, **5**, **6** and **7** to the pyruvate mixture **15** and **18** and subsequent hydrolysis to alcohols **20** and **21** established the structures of **6** and **7** by the same arguments applied for **4** and **5**.

Tetraludin H (**8a**), $C_{23}H_{28}O_{10}$, mp 172–173°, which

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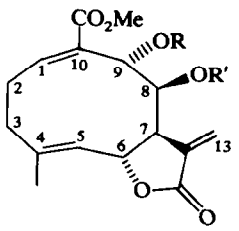
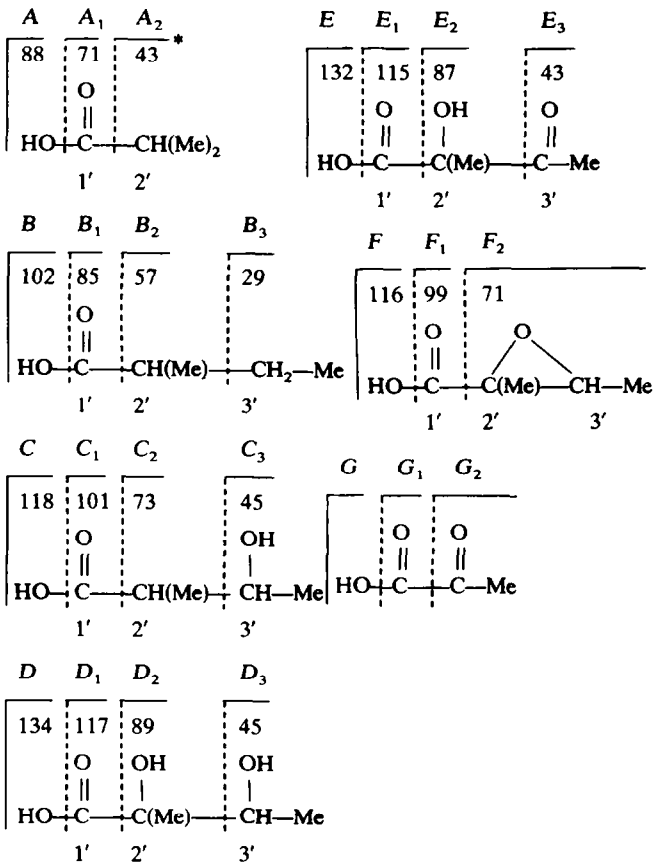
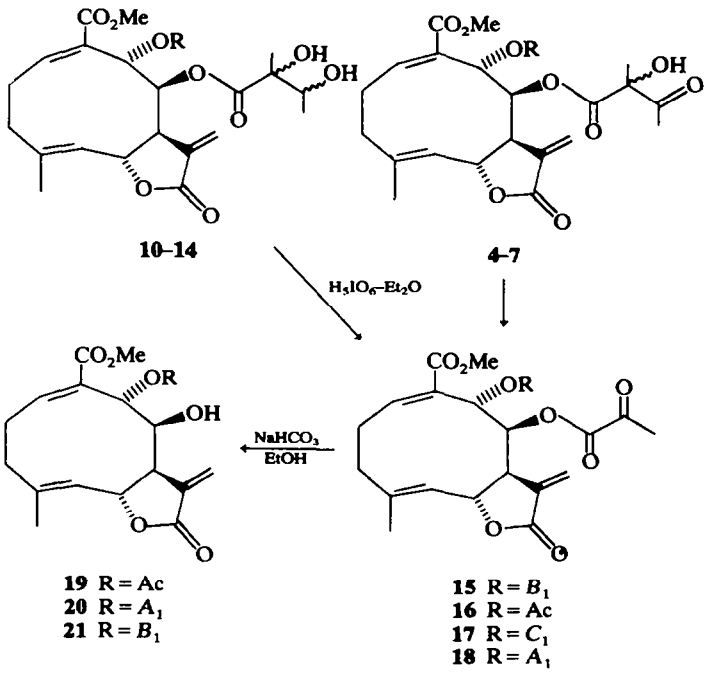


Name	R	R′	Comment
Tetraludin A (1)	Ac	D_1	
B (2)	C_1	D_1	epimeric at C-2′
C (3)	C_1	D_1	and/or C-3′ of D_1
D (4)	B_1	E_1	epimeric at C-2′
E (5)	B_1	E_1	of E_1
F (6)	A_1	E_1	epimeric at C-2′
G (7)	A_1	E_1	of E_1
H (8a)	Ac	E_1	C-2′ epimer of polydalin
(8b)	Ac	E_1	polydalin
I (9)	C_1	F_1	
J (10)	B_1	D_1	epimeric at C-2′
K (11)	B_1	D_1	and/or C-3′ of D_1
L (12)	A_1	D_1	epimeric at C-2′
M (13)	A_1	D_1	and/or C-3′ of D_1
N (14)	C_1	E_1	
(15)	B_1	G_1	
(16)	Ac	G_1	
(17)	C_1	G_1	
(18)	A_1	G_1	
(19)	Ac	H	
(20)	A_1	H	
(21)	B_1	H	

* Indicates observed MS fragmentations (see Table 3).





Scheme 1. Oxidation–Hydrolysis of tetraludins.

Table 1. Physical data of tetraludins A–N*

Compound	Empirical formula	mp	CD, c	CD, λ_{max}^{MeOH}, nm([θ])	IR, ν_{max} cm^{-1}
1	$C_{23}H_{30}O_{10}$	gum	1.6×10^{-4}	213(−4.4×10^4), 260(−1.2×10^3)	3500, 1760, 1740, 1715, 1665, 1625
2	$C_{26}H_{36}O_{11}$	164–165	8.0×10^{-5}	214(−1.0×10^5), 260(−2.6×10^3)	3450, 1765, 1740, 1715, 1665, 1620
3	$C_{26}H_{36}O_{11}$	172–173	3.5×10^{-5}	214(−1.2×10^5), 260(−3.6×10^3)	3500, 1765, 1735, 1715
4	$C_{26}H_{34}O_{10}$	139–140	6.5×10^{-5}	215(−7.6×10^4), 265(−1.6×10^3)	3500, 1765, 1735, 1725, 1720 sh
5	$C_{26}H_{34}O_{10}$	132–133	6.5×10^{-5}	212(−1.1×10^5), 265(−3.1×10^3)	3520, 1760, 1735, 1722
8a	$C_{23}H_{28}O_{10}$	172–173	1.0×10^{-4}	215(−1.1×10^4), 270(−2.3×10^3)	3500, 1760, 1735, 1720, 1705
9	$C_{26}H_{34}O_{10}$	gum	2.6×10^{-4}	226(−4.8×10^4), 265(−2.7×10^3)	3530, 1760, 1725, 1715, 1710
10	$C_{26}H_{36}O_{10}$	171–172	6.5×10^{-5}	215(−8.3×10^4), 260(−1.6+10^3)	3520, 1760, 1735, 1720, 1715
11	$C_{26}H_{34}O_{11}$	gum	1.2×10^{-4}	224(−1.1×10^5), 270(−5×10^3)	3500, 1760, 1730 sh, 1720, 1715 sh

* UV spectra run in MeOH showed strong end absorptions.

co-occurred with polydalin (**8b**) [2] exhibited ^{1}H NMR signals in C_6D_6 solution that were distinctly different from **8b** again suggesting a pair of positional isomers or structural isomers at C-2′ of side chain *E* in **8a** and **8b**. Periodate oxidation of a mixture of **8a** and **8b** provided compound **16** which was identical with the pyruvate that had been previously obtained from **1** [1], suggesting that **8a** and **8b** differ by opposite chirality at C-2′ of E. Tetraludin H was also shown to be identical with the oxidation product of tetraludin A (**1**). Since **1** was shown to bear an acetate function at C-9 [1] this correlation verified the structural assignment for **8a**.

Tetraludin I (**9**), $C_{26}H_{34}O_{10}$, exhibited ^{1}H NMR and MS patterns that indicated the same medium ring skeleton as the other tetraludins but contained the ester side chains 3-hydroxy-2-methylbutyrate (C_1) and epoxyangelate (F_1). The positions of the two ester moieties could not be established but by correlation with the other tetraludins which have the 2′–3′-dioxygenated ester moiety at C-8 we tentatively assign structure **9** to tetraludin I.

Tetraludin J (**10**), $C_{26}H_{36}O_{10}$, mp 171–172°, showed ^{1}H NMR and MS parameters which indicated 2-methyl-2,3-dihydroxybutyrate (D_1) and 2-methylbutyrate (B_1) moieties in the molecule. The relationships of **10** and **11**, **12** and **13** were the same as previously described for the tetraludin D–G series. Periodate oxidation of a mixture of **10–13** provided a mixture of **15** and **18** with 2-methylbutyrate signals, a doublet (J = 7 Hz) at 1.01 and a triplet (J = 7 Hz) at 0.75 and isobutyrate methyl doublets (J = 7 Hz) at 1.03 and 1.04 plus a pyruvate methyl singlet at 2.39. Selective saponification with $NaHCO_3$ of the mixture of **15** and **18** resulted in a mixture of alcohols **20** and **21** with a C-8 proton absorption centered at 5.17 suggesting the presence of the OH group at C-8 in **20** and **21** and therefore the attachment of the five-carbon ester D_1 to C-8 in **10–13** and B_1 in **10** and **11** as well as A_1 in **12** and **13** to C-9.

Table 2. ^{1}H NMR data of tetraludins D–N and derivatives*

Compound	**4**	**5**‡	**8a**	**8b**	**9**	**10**‡	**14**	**15**	**20**	Multiplicity (*J*-values in Hz)
H-1	6.96 (6.6)†	6.97 (6.69)	7.00 (6.70)	7.00 (6.71)	7.00 (6.72)	6.96 (6.70)	6.97 (6.72)	7.01	6.96	*dd*(10,8)
H-5	(4.46)	4.90 (4.50)	4.91 (4.51)	4.91 (4.49)	4.93 (4.53)	4.94 (4.54)	(4.53)	4.94	4.90	*brd* (10)
} 4.8–5.1						} 4.8–5.1				
H-6	(4.95)	5.09 (5.17)	5.18 (5.16)	5.07 (4.96)	5.14 (5.15)	5.10 (5.09)	(5.00)	5.18	5.23	*t*(10)
H-8	6.60 (6.81)	6.59 (6.82)	6.58 (6.82)	6.61 (6.85)	6.67 (6.90)	6.63 (6.85)	6.57 (6.83)	6.69	} 5.17	*dd* (8.5,1.5)
H-9	5.25 (5.31)	5.32 (5.39)	5.35 (5.41)	5.29 (5.35)	5.28 (5.36)	5.38 (5.39)	5.32 (5.40)	5.36		*d*(8.5)
H-13a	6.26 (6.18)	6.23 (6.20)	6.27 (6.21)	6.28 (6.21)	6.30 (6.22)	6.28 (6.24)	6.25 (6.23)	6.27	6.33	*d*(3.5)
H-13b	5.76 (5.66)	5.70 (5.65)	5.71 (5.66)	5.77 (5.69)	5.78 (5.70)	5.75 (5.70)	5.75 (5.70)	5.76	5.61	*d*(3.2
C-4-Me	1.95 (1.71)	1.98 (1.76)	1.97 (1.76)	1.97 (1.73)	2.00 (1.72)	2.00 (1.73)	1.95 (1.75	2.02	1.95	*d*/*brs*(1.2)
C-2′-Me	1.50 (1.29)	1.47 (1.41)	1.48 (1.40)	1.50 (1.30)	1.49 (1.34)	1.19 (1.05)	1.50 (1.47)	2.40	—	*s*
C-3′-Me	2.14 (1.77)	2.15 (1.89)	2.16 (1.90)	2.16 (1.79)	1.24 (1.05)	1.18 (1.03)	2.16 (1.88)	—	—	*d*/*s*(6.5)
H-3′	— —	— —			3.00 (2.51)	3.85 (3.79)	— —	—	—	*q*(6.5)
C-2″-Me	1.40 (0.97)	1.04 (0.94)			1.06 (0.89)	1.06 (1.01)	1.02 (0.87)	1.02	1.10	*d*(7)
C-3″-Me	0.82 (0.74)	0.82 (0.70)			1.15 (0.94)	0.82 (0.76)	1.15 (0.97)	1.06	0.88	*d*/*t*(7)
H-3″					4.88 (3.70)		3.80 (3.78)			*m*
H-2″	2.18 *sext*	2.16 *sext*			2.33 (2.22)		(2.28)	2.32		*m*
COOMe	3.78 (3.42)	3.78 (3.44)	3.79 (3.41)	3.79 (3.41)	3.79 (3.45)	3.80 (3.44)	4.78 (3.46)	3.79	3.75	*s*
OAc	— —	— —	1.97 (1.61)	1.97 (1.67)	— —	— —	— —	—	—	—

* Run at 100 MHz in $CDCl_3$ with TMS as internal standard. Values are in ppm (δ) relative to TMS.

† Values in parentheses are δ values determined in C_6D_6.

‡ Signals due to the two C-2′-Me of the isobutyrate moiety in $CDCl_3$ appeared at δ 1.08 and 1.06 (*d*, J = 7.0 Hz) in **6** and **7**, at 1.03 and 1.04 (*d*, J = 7.0 Hz) in a mixture of **15** and **18** and a mixture of **10**, **11**, **12** and **13**, respectively.

Table 3. Mass spectral data due to fragmentations

	4		6 and 7		8a and 8b	
M^+	506†(6.6)‡		492(3.2)		464(2.2)	
M−R−OH	—		—		—	
$M-R_1OH$	375(3.7)	M−*E*−H	361(5.3)	M−*E*−H	333(9.1)	M−*E*−H
R_1-fragments	131(4.3)	*E*−*H*	131(5.3)	*E*−*H*	131(10.2)	*E*−H
	115(3.6)	E_1	115(2.1)	E_1	115(11)	E_1
	113(1.2)	$E-H-H_2O$	113(2.1)	$E-H-H_2O$	113(2.4)	$E-H-H_2O$
	97(7.0)	E_1-H_2O	97(10)	E_1-H_2O	97(24)	E_1-H_2O
	87(9.0)	E_2	87(2.1)	E_2	87(25.4)	E_2
	69(11.2)	E_2-H_2O	69(6.3)	E_2-H_2O	69(25)	E_2-H_2O
	43(17.3)	E_3				
R-fragments	101(2.0)	*B*−*H*	87(2.1)	*A*−*H*	43	Ac
	85(59)	B_1	71(16)	A_1		
			43(16)	A_2		
Miscellaneous	464(9.1)	$M-CH_2CO$	450(4.2)	$M-CH_2CO$	422(17.6)	$M-CH_2CO$

* MS data were obtained at 70 eV by a direct inlet probe.
† Numbers denote observed *m/e* values for fragment ions.
‡ Numbers in parentheses denote relative intensities of observed ions.

Tetraludin N (**14**), $C_{26}H_{34}O_{11}$, exhibited ^{1}H NMR and MS signals that indicated the presence of 2-methyl-3-hydroxybutyrate (C_1) and 2-methyl-2-hydroxy-3-ketobutyrate (E_1). Periodate oxidation of **14** provided the pyruvate (**17**) which was identical with the degradation product obtained from tetraludin B (**2**) [1]. Selective hydrolysis of compound **17** with $NaHCO_3$ in ether had previously been established that side chains E_1 and C_1 are attached to C-8 and C-9, respectively, thus showing that structure **14** can be assigned to tetraludin N.

The above data clearly demonstrate that ester side chains with chiral centers that are attached to a terpenoid skeleton may vary in the chirality of the side chain at one center or more. The commonly used physical methods (IR, MS and also ^{1}H NMR in $CDCl_3$) may not detect the presence of a diastereomeric mixture and might suggest a pure compound. In the tetraludin series obtaining ^{1}H NMR spectra in C_6D_6 caused the diastereomeric pairs of melampolides to exhibit well-separated signals most dramatically for the proton absorptions due to the ester side chains which differed in chirality.

EXPERIMENTAL

Tetragonotheca ludoviciana (T. and G.) Gray; was collected in August 1977 at Tarrant Co., Texas (Bacon and Bragg 1652, voucher at The University of Texas at Arlington). Dried leaves (582 g) were extracted and worked up as previously described [3], providing 9.0 g of crude syrup which was chromatographed over 250 g Si gel using $CHCl_3$ and mixtures of $CHCl_3$–Me_2CO (2.5, 5.0, 10.0, 20.0, 40.0 and

Table 4. Mass spectral fragments of the medium ring portion of tetraludins*

m/e	Assignments	4	6 and 7	8a and 8b	9	10	12	14
362	$C_{19}H_{22}O_7$	14.9†	12.6	16.0	2.2	14.8	17.8	25.6
291	$C_{16}H_{18}O_5$	34.8	31.6	39.9	19.4	29.3	27.5	82.7
274	$C_{16}H_{18}O_4$	22.6	21.1	26.2	11.1	16.8	16.1	32.1
273	$C_{16}H_{17}O_4$	56.5	49.5	57.9	59.4	41.2	40.0	100.0
272	$C_{16}H_{16}O_4$	100.0	100.0	100.0	100.0	100.0	100.0	67.5
259	$C_{15}H_{15}O_4$	32.0	27.4	55.3	33.9	25.3	26.7	59.6
258	$C_{15}H_{14}O_4$	14.0	4.2	17.9	10.0	13.7	13.1	15.2
242	$C_{15}H_{14}O_3$	19.7	11.6	24.9	7.8	10.2	9.2	23.4
241	$C_{15}H_{13}O_3$	25.8	21.1	29.8	25.6	15.1	10.6	46.0
240	$C_{15}H_{12}O_3$	24.2	20.0	28.9	27.8	20.7	16.4	21.4
214	$C_{14}H_{14}O_2$	14.8	11.6	20.4	10.0	10.8	11.1	19.9
213	$C_{14}H_{13}O_2$	45.6	14.1	67.4	56.1	40.2	36.7	58.8
212	$C_{14}H_{12}O_2$	24.2	22.1	34.4	27.8	22.4	21.7	25.1

* MS data were obtained at 70 eV by direct inlet probe.
† Numbers denote relative intensities of observed ion peaks.

involving the ester side chains of the tetraludins*

9		10		12		14	
506(6.1)		508(2.1)		494(2.8)		522(3.8)	
388(5)	M−*C*					405(4.8)	M−*C*−H
—		375(2.7	M−*D*−H	361(2.8)	M−*D*−H	319(4.9)	M−*E*−H
115(2.8)	*F*−H	133(6.5)	*D*−H	133(7.8)	*D*−H	131(9.5)	*E*−H
99(18.3)	F_1	117(3.4)	D_1	117(4.4)	D_1	115(5.4)	E_1
71(15.6)	F_2	115(2.8)	$D-H-H_2O$	115(5.0)	$D-H-H_2O$	113(1.4)	$E-H-H_2O$
		99(2.7)	D_1-H_2O	99(1.4)	D_1-H_2O	97(5.5)	E_1-H_2O
		89(17.6)	D_2	89(19.4)	D_2	87(7.1)	E_2
		81(13.0)	D_1-2H_2O	81(10)	D_1-2H_2O	69(3.3)	E_2-H_2O
		71(18.4)	D_2-H_2O	71(33)	D_2-H_2O		
		53(4.8)	D_2-2H_2O	53(2.8)	D_2-2H_2O		
101(16.1)	C_1	101(1.6)	*B*−H	87(1.7)	*A*−H	117(5.5)	*C*−H
100(45.6)	$C-H_2O$	85(81)	B_1	71(33)	A_1	101(8.3)	C_1
99(7.2)	$C-H-H_2O$	57(84)	B_2	43(14)	A_2	99(1.5)	$C-H-H_2O$
83(18.3)	C_1-H_2O					83(11.5)	C_1-H_2O
55(17.8)	C_2-H_2O					55(4.4)	C_2-H_2O
490(2.2)	M−16	43(18)	C_3H_7			480(2.4)	$M-CH_2CO$
						391(4.9)	M−(*E*−H)
						373(3.7)	$M-(E-H-H_2O)$

80.0%) as eluant; 250 ml fractions were taken and all fractions were monitored by TLC.

Fractions 16–17, 18–19 and 22–24 contained **1**, **2** and **3**, respectively [1]. Rechromatography of less-popular fractions permitted the isolation of the new tetraludins. Fraction **11** (150 mg) when rechromatographed on 30 g Si gel using mixtures of petrol–Et_2O (5, 10, 20, 40, 80% Et_2O) as eluant (25 ml fractions), provided after further purification by TLC 40 mg of pure **4**. Subsequent fractions contained a mixture of **4** and **5** (25 mg) followed by mixtures of **6** and **7** (30 mg). Later fractions contained mixtures (30 mg) of **8a** and **8b** and **9** which were further separated by PLC. Rechromatography of fractions 12 and 13 of the original chromatographic run over Si gel and subsequent PLC separations gave pure **10** (8 mg) plus mixtures (25 mg) of **10** and **12** as well as **11** and **13**. Fractions **14** and **15** of the first CC run provided after repeated TLC separations pure **14** (20 mg) The physical data of the new compounds are summarized in Tables 1–4.

Periodate oxidations and selective hydrolyses. A mixture (20 mg) of tetraludins D–G (**4–7**) was treated with H_5IO_6 in Et_2O and worked up as previously described [1] to give a mixture (12 mg) of **15** and **18**. Periodate oxidation of a mixture (15 mg) of **8a** and **8b** gave **16** (8 mg) which was identical by ^1H NMR and IR with the pyruvate derivative derived from **1** [1]. A mixture of (12 mg) of tetraludins J–M (**10–13**) gave upon H_5IO_6 oxidation a mixture of **15** and **18**. Hydrolysis with $NaHCO_3$ by our previously described method [1] provided a mixture (5 mg) of **20** and **21**. Periodate oxidation of **14** (15 mg) resulted in **17** (10 mg) with ^1H NMR parameters identical with the product obtained from **2** [1].

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