Celenamides A and B, Linear Peptide Alkaloids[1] from the Sponge *Cliona celata*

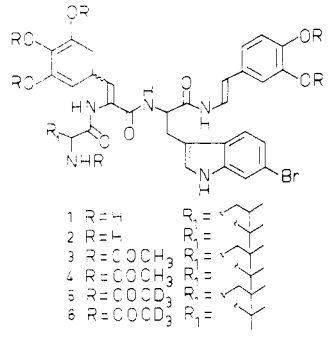
Richard J. Stonard and Raymond J. Andersen*

Departments of Oceanography and Chemistry, The University of British Columbia, Vancouver, British Columbia, Canada V6T 1W5

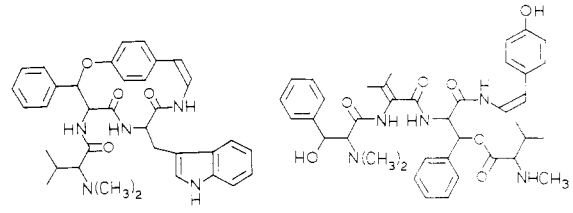
Received January 16, 1980

Two novel linear peptide alkaloids have been isolated as their hexaacetyl derivatives from acetylated methanol extracts of the sponge *Cliona celata*. The structures of hexaacetylcelenamide A (**3**) and B (**4**) were established by spectroscopic analysis and chemical degradation by ozonolysis and acid-catalyzed hydrolysis. The unique amino acid α,β-didehydro-3,4,5-trihydroxyphenylalanine was shown to be a subunit of both **3** and **4**. A small-scale isolation employing acetic-d_6 anhydride as the acetylating agent indicated that celenamides A (**1**) and B (**2**) were the naturally occurring sponge metabolites.

Marine sponges have been a rich source of unique amino acid derived metabolites.[5] In spite of this, sponges, like other marine organisms, rarely contain metabolites representative of the alkaloids found in terrestrial sources.[6] We now report the identification of two brominated linear peptide alkaloids[7] present in the sponge *Cliona celata* (Grant) which bear close resemblance to integerrin (**7**)[8] and lasoidine-A (**8**),[9] two recently reported plant metabolites. The structures of celenamide A (**1**) and celenamide B (**2**)

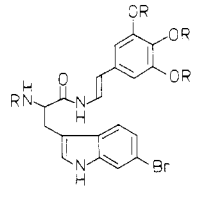


were deduced from spectroscopic and chemical studies of their hexaacetyl derivatives **3** and **4**. The celenamides are closely related to clionamide (**10**) which we have previously isolated from the same sponge.[10]



7 8

Cliona celata was collected by using SCUBA in Howe Sound, British Columbia, Canada (−20 m), and La Jolla, CA (−40 m). Methanol extracts of the homogenized sponge were acetylated with acetic anhydride in pyridine. Silica gel column chromatography of the acetylated residue gave two major fractions: one containing a mixture of acetylcelenamides and the second containing tetraacetylclionamide (**9**). The acetylcelenamide-containing fraction was



9 R = COCH3
10 R = H

subjected to repeated preparative layer chromatography on silica gel followed by fractionation on reversed-phase plates (Whatman KC_{18}) to yield hexaacetylcelenamides A (**3**, $[\alpha]^{25}_D$ +40° (*c* 1.1, acetone), 0.03% wet weight) and B (**4**, $[\alpha]^{25}_D$ +22° (*c* 1.1, acetone), 0.02% wet weight) as noncrystalline white solids. The peptidyl nature of **3** and **4** was evident from intense amide absorptions in the infrared spectrum [ν_{max} 3300 (NH stretch), 1660 cm^{-1} (amide I band)] and two peptide α-methine signals present in the ^1H NMR spectrum of each compound Table I).

Molecular ions were not observed in the electron-impact mass spectra (10–70 eV) of hexaacetylcelenamides A (**3**) and B (**4**). The elemental compositions of $C_{46}H_{48}BrN_5O_{14}$ and $C_{45}H_{46}BrN_5O_{14}$ were inferred from ^1H NMR (**3**, 43 CH and 5 NH; **4**, 41 CH and 5 NH) and mass spectra [**3** and

(1) The classical definition of alkaloids demands that they be isolated from plants.[2] Salamander and arthropod alkaloids are well-established exceptions to this classification.[3] A more contemporary definition would be that an alkaloid is any naturally occurring, nitrogen-containing compound which is basic.[4] Celenamides A and B are not of plant origin but they qualify as alkaloids because they fit all the criteria of the above stated contemporary definition, and they are obvious members of the well-established peptide alkaloid family.[7]

(2) Pelletier, S. W. "Chemistry of the Alkaloids"; Van Nostrand-Reinhold: New York, 1970.

(3) (a) Habermehl, G. In "The Alkaloids"; Manske, R. H. F., Ed.; Academic Press: New York, 1967; Vol. IX, Chapter 9. (b) Tursch, B.; Braekman, J. C.; Daloze, D. *Experientia* **1976**, *32*, 401–536.

(4) Robinson, T. "The Biochemistry of Alkaloids"; Springer-Verlag: New York, 1968.

(5) (a) For example, see: Fattorusso, E.; Minale, L.; Sodano, G. *J. Chem. Soc., Perkin Trans. 1* **1972**, 16–8. (b) Moody, K.; Thomson, R. H.; Fattorusso, E.; Minale, L.; Sodano, G. *Ibid.* **1972**, 18–24. (c) Charles, E.; Braekman, J. C.; Daloze, D.; Tursch, B.; Karlsson, R. *Tetrahedron Lett.* **1978**, 1519–20. (d) Kazlauskas, R.; Murphy, P. T.; Wells, R. J. *Tetrahedron Lett.* **1978**, 4945–8. (e) Minale, L.; Cimino, G.; De Stefano, S.; Sodano, G. *Fortschr. Chem. Org. Naturst.* **1976**, *33*, 1–72.

(6) A series of tryptamines have been isolated from sponges. See: (a) Van Lear, G. E.; Morton, G. O.; Fulmor, W. *Tetrahedron Lett.* **1973**, 299; (b) Djura, P.; Stierle, D. B.; Sullivan, B.; Faulkner, D. J.; Arnold, E.; Clardy, J. *J. Org. Chem.* **1980**, *45*, 1435.

(7) (a) For a definition of peptide alkaloid and an excellent review of the family, see: Pais, M.; Jarreau, F.-X. In "Chemistry and Biochemistry of Amino Acids, Peptides and Proteins"; Weinstein, B., Ed.; Marcel Dekker: New York, 1971; Vol. 1, Chapter 5. (b) Tschesche, R.; Kaussman, E. U. In "The Alkaloids"; Manske, R. H. F., Ed.; Academic Press: New York, 1975; Vol. XV, p 165.

(8) Tschesche, R.; Frohberg, E.; Fehlhaber, H.-W. *Tetrahedron Lett.* 1968, 1311.

(9) Marchand, J.; Pais, M.; Jarreau, F. *Tetrahedron* **1969**, *25*, 937-54.

(10) (a) Andersen, R. J. *Tetrahedron Lett.* **1978**, 2541–4. (b) Andersen, R. J.; Stonard, R. J. *Can. J. Chem.* **1979**, *57*, 2325–8.

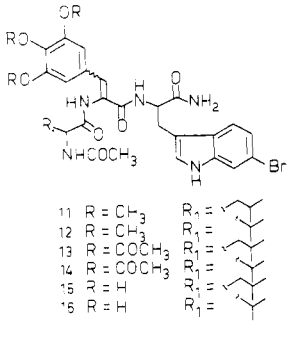
0022-3263/80/1945-3687$01.00/0

Table I. 270-MHz ^{1}H NMR Spectra[a]

proton position[m]	chemical shift[b] 9	3	4	13	14
indole H_1	10.22 (s)	10.18 (s)	10.19 (s)	10.16 (s)	10.24 (s)
H_2[c]	7.20	7.31	7.34	7.26	7.33
H_4[d]	7.59	7.62	7.64	7.64	7.62
H_5[e]	7.16	7.16	7.19	7.15	7.17
H_7[f]	7.57	7.57	7.59	7.58	7.60
Trp NH	7.41 (d, 8)	7.91 (d, 10)	7.76 (d, 10)[g]	7.81 (d, 8)	7.65 (d, 8.5)
CH_2	3.28 (dd, 6.3, 15)	3.54 (dd, 4, 15)	3.48 (dd, 15.4, 4.5)	3.51 (dd, 4, 15)	3.44 (dd, 4.3, 15.4)
	3.14 (dd, 7, 15)	3.26 (dd, 11, 15)	3.29 (dd, 15.4, 11)	3.25 (dd, 11.5, 15)	3.16 (dd, 10, 15.4)
CH	4.77 (ddd, 7, 8, 6.3)	4.75 (ddd, 11, 4, 10)	4.68 (ddd, 10, 11, 4.5)	4.66 (ddd, 11.5, 4, 8)	4.71 (ddd, 10, 4.3, 8.5)
Leu CH		4.32 (m)		4.28 (m)	
CH_2		1.73 (m)		1.67 (m)	
Val CH			4.46 (dd, 6, 8.2)		4.39 (dd, 6, 8.5)
i-Pr CH		1.86 (m)	2.24 (m)	1.87 (m)	2.02 (m)
$(CH_3)_2$[l]		0.97, 0.99	0.99, 1.01	0.95, 0.99	0.97, 0.99
NHAc	1.91 (s)	1.97 (s)	1.91 (s)	1.98 (s)	1.86 (s)
OAc	2.26 (s, 6 H)	2.28 (s, 9 H)	2.29 (s, 3 H)	2.28 (s, 9 H)	2.23 (s, 6 H)
	2.27 (s, 3 H)	2.27 (s, 3 H)	2.25 (s, 3 H)		2.29 (s, 3 H)
		2.25 (s, 3 H)	2.24 (s, 9 H)		
NHCH=CH[h]	7.46	7.50	7.49		
NHCH=CH[i]	6.14	6.52	6.40		
NHCH=CH[j]	9.54	9.50	9.54		
phenyl H	7.13 (s, 2 H)	7.17 (s, 2 H)	7.41 (s, 2 H)	7.21 (s, 2 H)	7.42 (s, 2 H)
		7.15 (d, 9, 1 H)	7.15 (d, 9, 1 H)		
		7.24 (d, 2, 1 H)	7.21 (d, 2, 1 H)		
		7.23 (dd, 2, 9, 1 H)	7.18 (dd, 2, 9, 1 H)		
CO(NHR)C=CH		6.79 (s)	7.16 (s)	6.88 (s)	7.22 (s)
CO(NHR)C=CH		9.42 (s)	9.47 (s)	9.33 (s)	9.46 (s)
$CONH_2$				6.51, 7.21[k]	6.54, 7.22[k]
Val NH			7.80 (d, 6)[g]		7.82 (d, 6)
Leu NH		7.93 (d, 5)		7.91 (d, 5.5)	

[a] All ^{1}H NMR spectra were recorded in $(CD_3)_2CO$. [b] In parts per million from internal tetramethylsilane. Multiplicities, coupling constants (J, in hertz), and the number of hydrogen atoms are given in parentheses. [c] d, J = 2.2 Hz. [d] d, J = 8.8 Hz. [e] dd, J = 1.4, 8.8 Hz. [f] d, J = 1.4 Hz. [g] Assignments may be interchanged. [h] dd, J = 10.0, 15.0 Hz. [i] d, J = 15.0 Hz. [j] d, J = 10.0 Hz. [k] 1 H, singlets. [l] d, J = 7.0 Hz. [m] Assignments were made by extensive decoupling and D_2O-exchange experiments.

4 displayed the fragment ion $C_{12}H_{13}NO_4^{+\cdot}$ for C-terminal diacetoxystyrylamine[7] (see Figure 1)] in conjunction with the molecular formulas, $C_{31}H_{38}BrN_5O_7$ and $C_{30}H_{36}BrN_5O_7$, of their methylated hydrolysis products 11 and 12 determined from exact mass measurements.



11 R = CH_3 R_1 =
12 R = CH_3 R_1 =
13 R = $COCH_3$ R_1 =
14 R = $COCH_3$ R_1 =
15 R = H R_1 =
16 R = H R_1 =

The ^{1}H NMR spectrum of peptide 3 (Table I) displayed resonances at δ 6.52 (d, 1 H, J = 15 Hz), 7.50 (dd, 1 H, J = 10, 15 Hz), and 9.50 (d, 1 H, J = 10 Hz, exchangeable) indicative of a trans substituted enamide.[9,10] The observation of intense fragment ions at m/e 235, 193, and 151 in the mass spectrum (Figure 1) suggested that the enamide substituent was a diacetoxyphenyl group.[7] Evidence for the same residue was apparent in the spectral data of peptide 4. Ozonolysis of enamides 3 and 4 in methanol at −78 °C followed by reduction of the ozonides with dimethyl sulfide yielded equimolar amounts of 3,4,5-triacetoxybenzaldehyde and 3,4-diacetoxybenzaldehyde plus polar products resulting from ozonolysis of the indole ring. The production of 3,4-diacetoxybenzaldehyde in this reaction is consistent with the presence of (*E*)-1-amino-2-(3,4-diacetoxyphenyl)ethene as the C-terminal residue of both hexaacetylcelenamides A (3) and B (4).

Treatment of enamides 3 and 4 with 0.1 N hydrochloric acid in acetonitrile at room temperature for 24 h gave the primary amides 13 and 14.[11] The richly detailed ^{1}H NMR spectra of these products revealed a number of structural features. Two peptide α-methine protons were observed for each molecule (13, δ 4.28 and 4.66; 14, δ 4.39 and 4.71), and both showed uncoupled olefinic signals (13, δ 6.88; 14, δ 7.22) and highly deshielded NH singlets (13, δ 9.33; 14, δ 9.46) characteristic of phenyl-substituted dehydroamino acid residues.[12] Also discernible were an acetamide resonance (13, δ 1.98; 14, δ 1.86) and two primary amide hydrogens (13, δ 6.51, 7.21; 14, δ 6.54, 7.22), indicating that 13 and 14 were tripeptides with the N terminus acetylated and the C terminus protected as a primary amide.

The nature of the two α-amino acid residues was apparent from the spectral data of hexaacetylcelenamides A (3) and B (4). An abundant fragment ion at m/e 210/208, characteristic of bromoindole derivatives,[10] was observed in the mass spectra, and comparison of the UV (λ_{max} 289 nm) and ^{1}H NMR spectra (Table I) with the corresponding spectra of tetraacetylclionamide (9; UV λ_{max}

(11) Katner, A. *J. Org. Chem.* **1961**, *26*, 3495–8.
(12) Morgenstern, A. P.; Schutij, C.; Nauta, W. T. *J. Chem. Soc. D* **1969**, 321–2.

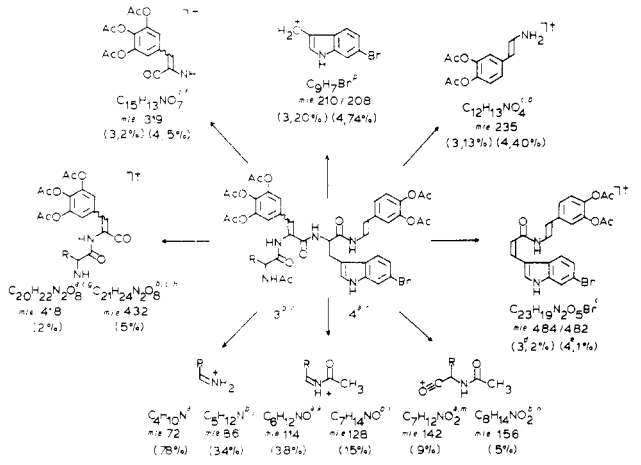
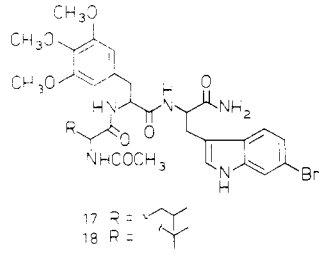


Figure 1. Interpretation of the mass spectra of **3** and **4** (see q below): a, R = $CH(CH_3)_2$; b, R = $CH_2CH(CH_3)_2$; c, consecutive losses of C_2H_2O are observed from these ions (see Experimental Section); d, exact mass measurement obtained on daughter ions only ($-C_2H_2O$); e, exact mass measurement not obtained; f, mol wt calcd 319.0692, obsd 319.0671; g, mol wt calcd 418.1377, obsd 418.1388; h, mol wt calcd 432.1533, obsd 432.1548; i, mol wt calcd 72.0812, obsd 72.0812; j, mol wt calcd 86.0970, obsd 86.0978; k, mol wt calcd 114.0919, obsd 114.0915; l, mol wt calcd 128.1075, obsd 128.1077; m, mol wt calcd 142.0868, obsd 142.0872; n, mol wt calcd 156.1025, obsd 156.1026; o, mol wt calcd 235.0845, obsd 235.0839; p, mol wt calcd 207.9761, obsd 207.9763; q, fragment ion intensities vary with instrument conditions; r, base peak *m/e* 43.

290 nm) clearly indicated that this moiety was present as a 6-bromotryptophan unit. Strong ions at *m/e* 86 (34%) and 72 (78%) in the mass spectra and signals at high field in the ^1H NMR spectra corresponding to isopropyl groups [**3**, δ 0.97, 0.99; **4**, δ 0.99, 1.01 (each d, 3 H, J = 7 Hz)] suggested the presence of a leucine and valine residue in **3** and **4**, respectively. Hydrolysis of peptides **3** and **4** in 6 N hydrochloric acid at 100 °C for 18 h afforded leucine and valine as expected. All the required signals for the 6-bromotryptophan, leucine, and valine residues could be found in the ^1H NMR spectra of the hydrolysis products **13** and **14** (Table I).

The exact nature of the third amino acid residue, known to be a phenyl-substituted dehydroamino acid from the ^1H NMR data, was determined by chemical degradation. Ozonolysis of the primary amides **13** and **14** gave 3,4,5-triacetoxybenzaldehyde, indicating that an α,β-didehydro-3,4,5-trihydroxyphenylalanine residue must be present. Additional evidence for the dehydroamino acid was provided by the isolation of oxalic acid from an acid hydrolysate of the polar products obtained from the ozonolysis of **3** and **4**. Reduction of a small sample of a 1:1 mixture of the trimethylated hydrolysis products **11** and **12** with sodium borohydride gave in high yield a mixture of the tripeptides **17** and **18**. Sodium borohydride reductions are characteristic reactions of dehydroamino acids.[13]



We could conclude from the above data that tripeptide **13** contained 6-bromotryptophan, α,β-didehydro-3,4,5-trihydroxyphenylalanine, and leucine residues while tripeptide **14** contained 6-bromotryptophan, α,β-didehydro-3,4,5-trihydroxyphenylalanine, and valine residues. Hence, the structures of hexaacetylcelenamides A (**3**) and B (**4**) must consist of tripeptides **13** and **14** bearing C-terminal (*E*)-1-amino-2-(3,4-diacetoxyphenyl)ethene residues in place of the primary amides. The mild acid hydrolysis of the trans-substituted enamide in **3** and **4** generates the primary amide functionalities in **13** and **14**.

The amino acid sequences of hexaacetylcelenamides A (**3**) and B (**4**) were deduced from their mass spectra (Figure 1). In the mass spectra of most peptide alkaloids the strongest ions originate from the N-terminal amino acid.[7,14] Abundant fragment ions at *m/e* 86 (34%) and 72 (78%) suggested, therefore, that leucine and valine represented the N-terminal residues of **3** and **4**, respectively. This was confirmed by the presence of diagnostic ions observed at *m/e* 128 (15%) and 156 (5%) in the spectrum of **3** and at *m/e* 114 (38%) and 142 (9%) in the spectrum of **4**, originating from *N*-acetylleucine and *N*-acetylvaline. The 6-bromotryptophan and α,β-didehydro-3,4,5-trihydroxyphenylalanine residues were sequenced on the basis of two diagnostic fragment ions. Each of these fragment ions lead to a series of daughter ions resulting from losses of C_2H_2O typical of phenol acetates. The mass spectra of both **3** and **4** displayed an *m/e* 484/482 ion (Figure 1) resulting from the elimination of NHCOR and H which is commonly observed in tryptophan-containing metabolites.[10] The presence of this characteristic ion suggested that 6-bromotryptophan was linked to the C-terminal styrylamino group. Support for this hypothesis comes from the observation of *m/e* 432 and 418 ions in the spectra of **3** and **4**, respectively, indicating that the α,β-didehydro-3,4,5-trihydroxyphenylalanine residue was linked to the N-terminal leucine and valine residues. Hence, the structures of hexaacetylcelenamides A (**3**) and B (**4**) must be as shown.

The isolation of hexaacetylcelenamide-d_{18} A (**5**) and hexaacetylcelenamide-d_{18} B (**6**) from a small-scale workup employing acetic-d_6 anhydride as the derivatizing agent led to the conclusion that celenamides A (**1**) and B (**2**) were the naturally occurring sponge metabolites. The susceptibility of **1** and **2** toward air oxidation and the difficulties inherent in attempting to separate two polar metabolites which are so structurally similar have prevented us from isolating them in their underivatized forms.

Celenamide A and celenamide B represent two new members of the peptide alkaloid family. They are the first examples which have not been isolated from terrestrial plants, and while they show a striking resemblance to integerrin (**7**),[8] they contain a number of unique structural features. The 6-bromotryptophan residue and the new amino acid α,β-didehydro-3,4,5-trihydroxyphenylalanine are without precedent in this family of metabolites. Celenamide A, celenamide B, and clionamide all possess nonmethylated amino groups and C-terminal *trans*-styrylamino residues. All other peptide alkaloids possess the cis geometry at their C termini. The only other example of a linear peptide alkaloid is lasoidine A (**8**).[9]

Experimental Section

^1H NMR spectra were recorded on a Nicolet-Oxford 270 spectrometer. ^{13}C NMR spectra were recorded on Varian CFT-20

(13) Liesch, J. M.; Rinehart, K. L. *J. Am. Chem. Soc.* **1977**, *99*, 1645–6 and references therein.

(14) Lagarias, J. C.; Goff, D.; Klein, F. K.; Rapoport, H. *Llyodia* **1979**, *42*, 220–7.

and Bruker WP-80 spectrometers. Tetramethylsilane (δ 0) was used as an interal reference in all ^1H and ^{13}C NMR spectra. Low-resolution mass spectra and high-resolution mass spectra were recorded on AEI MS-902 and MS-50 spectrometers, respectively. Ultraviolet and infrared spectra were recorded on Cary 14 and Perkin-Elmer Model 710B spectrophotometers, respectively. Optical rotations were measured on a Perkin-Elmer Model 241C polarimeter using a 1-cm microcell. Gas chromatography was performed on a Hewlett-Packard 5830A instrument equipped with a thermal-conductivity detector.

Isolation of Hexaacetylcelenamides A (3) and B (4). The sponge *Cliona celata* (1 kg, wet weight) was collected and extracted, and the ethyl acetate soluble portion of the methanol extract was acetylated as previously described.[5] Chromatography of the acetylated residue on a column of silica gel G provided a fraction eluting with 20–40% ethyl acetate in chloroform and containing a mixture of **3** and **4** and a second fraction eluting with ethyl acetate and containing tetraacetylclionamide (**9**; 2.0 g, 0.13%, wet weight). Pure hexaacetylcelenamide A (**3**; 310 mg, 0.03%, wet weight) and hexaacetylcelenamide B (**4**; 240 mg, 0.02%, wet weight) were obtained as noncrystalline white solids by repeated chromatography on silica plates with chloroform–methanol (19:1) as eluant and reversed-phase chromatography on Whatman KC_{18} plates with water–methanol–tetrahydrofuran (3:5:2) as eluant.

A sample of *Cliona celata* collected near La Jolla, CA (35-m depth), also provided **3**, **4**, and **9** in similar yields.

Hexaacetylcelenamide A (3): $[\alpha]^{25}_D$ +40° (*c* 1.1, acetone); UV (MeOH) λ_{max} 227 nm (ϵ 72 000), 289 (br, ϵ_{max} 51 000); IR ($CHCl_3$) 3300 (br), 1780, 1660 (br), 1500 (br), 1380, 1200 cm^{-1}; mass spectrum (70 eV), *m/e* (peaks not shown in Figure 1, relative intensity, formula calculated by high-resolution mass spectrometry; deviation from calcd value is < ± 0.0030 amu) 43 (100), 99 (12, $C_4H_5NO_2$), 123 (6, $C_7H_7O_2$), 129 (13, C_9H_7N), 130 (10, C_9H_8N), 151 (30, $C_8H_9NO_2$), 165 (16, $C_8H_7NO_3$), 193 (20, $C_{10}H_{11}NO_3$), 193 (5, $C_9H_7NO_4$), 235 (7, $C_{11}H_9NO_5$), 235 (13, $C_{12}H_{13}NO_4$), 277 (6, $C_{13}H_{11}NO_6$), 306 (4, $C_{15}H_{13}N_2O_5$), 348 (7, $C_{17}H_{20}N_2O_6$), 390 (8, $C_{19}H_{22}N_2O_7$), 442/440 (1, $C_{21}H_{17}BrN_2O_4$), 501/499 (1), 543/541 (1).

Hexaacetylcelenamide B (4): $[\alpha]^{25}_D$ +22° (*c* 1.1, acetone); UV (MeOH) λ_{max} 227 nm (ϵ 80 000), 289 (br, ϵ_{max} 60 000); IR ($CHCl_3$) 3340 (br), 1780, 1660 (br), 1510 (br), 1380, 1200 cm^{-1}; mass spectrum (70 eV), *m/e* (peaks not shown in Figure 1, relative intensity, formula calculated by high-resolution mass spectrometry; deviation from calcd value is < ± 0.0030 amu) 43 (100), 99 (13, $C_4H_5NO_2$), 102 (62, C_8H_6), 123 (12, $C_7H_7O_2$), 129 (27, C_9H_7N), 130 (30, C_9H_8N), 151 (74, $C_8H_9NO_2$), 165 (20, $C_8H_7NO_3$), 193 (66, $C_{10}H_{11}NO_3$), 193 (2, $C_9H_7NO_4$), 235 (40, two signals overlap), 277 (9, $C_{13}H_{11}NO_6$), 292 (7, $C_{14}H_{16}N_2O_5$), 334 (7), 376 (7), 501/499 (1), 543/541 (1), 585/583 (1).

Hydrolysis of Hexaacetylcelenamides A (3) and B (4) with Hydrochloric Acid. To 200 mg of **3** was added 50 mL of acetonitrile and 0.5 mL of hydrochloric acid. The solution was stirred at room temperature for 24 h. Evaporation of the solvents gave an oil which was chromatographed on silica plates with chloroform–methanol (5:1) as eluant to give 21 mg of the primary amide **13**: mp 153–156 °C ($CHCl_3$–acetone); IR ($CH_3C{\equiv}N$) 3600, 3530, 3340, 1780, 1670 cm^{-1}; ^{13}C NMR ($(CD_3)_2CO$) δ 175.4, 174.6, 173.3, 168.5 (2 C), 167.6, 165.3, 144.6 (2 C), 133.0–112.2 (complex), 55.7, 54.6, 40.4, 33.2–26.9 (masked by acetone signal) 25.5, 23.4, 22.8, 21.8, 20.6 (2 C), 20.1.

Similar treatment of **4** (150 mg) gave the primary amide **14** (18 mg) as a noncrystalline white solid: IR ($CH_3C{\equiv}N$) 3600, 3530, 3340, 1780, 1670 cm^{-1}; ^{13}C NMR ($CD_3C{\equiv}N$) δ 175.2, 173.6 (2 C), 169.4 (2 C), 168.2, 165.4, 144.7 (2 C), 139.0–111.3 (complex), 60.3, 56.4, 31.1, 27.1, 23.0, 20.9 (2 C), 20.5, 19.7, 18.6.

Ozonolysis of Hexaacetylcelenamide A (3) and B (4). Ozone was passed through a methanol solution (40 mL) of **3** (32 mg) at −78 °C for 5 min. Excess ozone was removed in a stream of oxygen, dimethyl sulfide (2 mL) was added, and the mixture was allowed to warm to room temperature. Evaporation of the solvents and purification on silica plates with ethyl acetate as eluant gave 3,4,5-triacetoxybenzaldehyde (4 mg) and 3,4-diacetoxybenzaldehyde (3 mg) which were identified by comparison (TLC and IR, ^1H NMR, and mass spectra) with authentic samples prepared from gallic acid[5] and 3,4-dihydroxybenzaldehyde (Aldrich), respectively.

3,4,5-Triacetoxybenzaldehyde (3 mg) and 3,4-diacetoxybenzaldehyde (2 mg) were also obtained by ozonolysis of **4** (22 mg).

Ozonolysis of the Primary Amides 13 and 14. Treatment of **13** (10 mg) and **14** (10 mg) as described for the ozonolysis of **3** and **4** gave 3,4,5-triacetoxybenzaldehyde (2 mg each; TLC and ^1H NMR and mass spectra) as the sole benzaldehydic product.

Methanolysis of the Primary Amides 13 and 14. The primary amide **13** (6 mg) was dissolved in methanol (20 mL), and the solution was stirred at 50 °C for 4 h under an atmosphere of nitrogen. The reaction mixture was cooled to room temperature, and excess diazomethane in diethyl ether was added. The solvents were evaporated to obtain **11**: IR (KBr) 3340 (br), 2950, 1640 (br) cm^{-1}; ^1H NMR ($(CD_3)_2SO$) δ 0.92 (d, 1 H, *J* = 7 Hz), 0.98 (d, 1 H, *J* = 7 Hz), 1.7 (m, 3 H), 1.98 (s, 3 H), 3.33 (m, 2 H), 3.67 (s, 3 H, OMe), 3.78 (s, 6 H, OMe), 4.26 (m, 1 H), 4.61 (m, 1 H), 6.87 (s, 2 H), 7.06 (s, 1 H), 7.14 (dd, 1 H, *J* = 2, 8 Hz), 7.12 (br s, 1 H, exch), 7.28 (d, 1 H, *J* = 2 Hz), 7.40 (br s, 1 H, exch), 7.50 (d, 1 H, *J* = 2, 8 Hz), 7.60 (d, 1 H, *J* = 8 Hz), 8.33 (m, 2 H, exch), 9.96 (s, 1 H, exch), 10.90 (br s, 1 H, exch); mass spectrum (70 eV), *m/e* (relative intensity) 673/671 (1:1, 2.4), 266/264 (1:1, 16), 210/208 (1:1, 58), 181 (30), 130 (28), 129 (53), 128 (44), 86 (100), 43 (95); high-resolution mass measurement *m/e* 673.1927, $C_{31}H_{38}{}^{81}BrN_5O_7$ requires *m/e* 673.1934.

Treatment of **14** with methanol as described for **13** afforded the trimethoxy derivative **12**: IR (KBr) 3340 (br), 2950, 1640 (br) cm^{-1}; ^1H NMR ($(CD_3)_2SO$) δ 0.86 (d, 3 H, *J* = 6 Hz), 0.91 (d, 3 H, *J* = 6 Hz), 1.80 (s, 3 H), 1.97 (m, 1 H), 2.97 (dd, 1 H, *J* = 10, 15 Hz), 3.17 (dd, 1 H, *J* = 5, 15 Hz), 3.67 (s, 3 H, OMe), 3.78 (s, 6 H, OMe), 4.16 (dd, 1 H, *J* = 2, 8.4 Hz), 4.61 (ddd, 1 H, *J* = 5, 8.6, 10 Hz), 6.98 (s, 2 H), 7.09 (dd, 1 H, *J* = 2, 8.4 Hz), 7.11 (br s, 1 H, exch), 7.17 (s, 1 H), 7.22 (d, 1 H, *J* = 2 Hz), 7.40 (br s, 1 H, exch), 7.43 (d, 1 H, *J* = 8.6 Hz, exch), 7.51 (d, 1 H, *J* = 2 Hz), 7.62 (d, 1 H, *J* = 8.4 Hz), 8.36 (d, 1 H, *J* = 7 Hz, exch), 10.01 (s, 1 H, exch), 11.03 (br s, 1 H, exch); mass spectrum (70 eV), *m/e* (relative intensity) 659/657 (1:1, 0.4), 266/264 (1:1, 32), 210/208 (1:1, 100), 181 (57), 129 (40), 114 (27), 72 (52), 43 (52); high-resolution mass measurement *m/e* 659.1786, $C_{30}H_{36}{}^{81}BrN_5O_7$ requires *m/e* 659.1778.

The triphenolic compounds **15** and **16** were observed in ^1H NMR experiments in which $(CD_3)_2CO$ solutions of **13** and **14** were warmed (50 °C, 30 min) in the presence of water, D_2O, or CD_3OD. For **15**: ^1H NMR δ 0.91 (d, 3 H, *J* = 7 Hz), 0.98 (d, 3 H, *J* = 7 Hz), 1.7 (m, 3 H), 1.89 (s, 3 H), 3.31 (m, 2 H), 4.42 (m, 1 H), 4.72 (m, 1 H), 6.44 (br s, 1 H, exch), 6.72 (s, 2 H), 7.08 (s, 1 H), 7.16 (dd, 1 H, *J* = 2, 8.4 Hz), 7.21 (br s, 1 H, exch), 7.30 (d, 1 H, *J* = 2 Hz), 7.54 (d, 1 H, *J* = 2 Hz), 7.60 (d, 1 H, *J* = 8.4 Hz), 7.79 (s, 1 H, exch, ArOH), 7.83 (s, 2 H, exch, ArOH), 8.12 (m, 2 H, exch), 8.88 (s, 1 H, exch), 10.21 (br s, 1 H, exch). For **16**: ^1H NMR δ 0.94 (d, 3 H, *J* = 7 Hz), 0.97 (d, 3 H, *J* = 7 Hz), 1.9 (m, 1 H), 1.97 (s, 3 H), 3.3 (m, 2 H), 4.35 (m, 1 H), 4.78 (m, 1 H), 6.76 (s, 2 H), 7.14 (dd, 1 H, *J* = 2, 8 Hz), 7.20 (s, 1 H), 7.35 (br s, 1 H), 7.57 (d, 1 H, *J* = 2 Hz), 7.64 (d, 1 H, *J* = 8 Hz). Ferric chloride positive reactions were given by both **15** and **16**.

Hydrolysis of Hexaacetylcelenamides A (3) and B (4) with 6 N Hydrochloric Acid. A glass ampule containing 14 mg of **3** and 5 mL of 6 N hydrochloric acid was sealed under nitrogen and heated at 100 °C for 18 h. The ampule was cooled to room temperature and opened, and the contents were washed out with 5 mL of distilled water. The solvent was evaporated in vacuo, and the solid obtained was triturated with 1 N acetic acid. Filtration of the mixture and evaporation of the solvent in vacuo gave 1 mg of a light brown solid. Two-dimensional TLC (cellulose; first dimension, 1-butanol saturated with ammonia; second dimension, 1-butanol–acetic acid (9:1) saturated with water) indicated the presence of leucine.

Similar treatment of **4** with 6 N hydrochloric acid gave valine.

Oxalic Acid. The complex mixture of polar products (20 mg) obtained from the ozonolysis of **3** was dissolved in 3 N hydrochloric acid (10 mL), and the solution was refluxed for 1 h. The solvent was evaporated in vacuo, and the solid obtained was triturated with methanol. The methanol-soluble material was treated with excess diazomethane in diethyl ether, and the mixture was analyzed by gas chromatography (10% DEGS, 4 mm × 2 m column, He flow rate 30 mL/min). The sole peak observed in the gas chromatogram had an identical retention time (R_T = 4.61 min) with authentic dimethyl oxalate.

Dimethyl oxalate was also observed when the polar material obtained from the ozonolysis of 4 (14 mg) was treated in a similar fashion.

Reduction of the Enamides 11 and 12 with Sodium Borohydride. To 2 mg of a 1:1 mixture of 11 and 12 dissolved in 15 mL of ethanol was added 100 mg of sodium borohydride. The solution was stirred at room temperature for 20 h. Excess reagent was destroyed with 1 N hydrochloric acid, and the solution was concentrated and extracted with dichloromethane. The dichloromethane layer was dried over anhydrous sodium sulfate and evaporated to give a mixture of 17 and 18: ^{1}H NMR ($(CD_3)_2SO$) δ 1.0 (m, $(CH_3)_2CH$), 1.7 (m, Leu γ-CH and β-CH_2), 1.78 (s, NHAc), 1.9 (m, Val β-CH), 2.73 (dd, J = 7.5, 15.7 Hz, Trp β-CH_2), 2.90 (dd, J = 6.3, 15.7, Trp β-CH_2), 3.36 (s, OMe), 3.66 (m, β-CH_2), 3.93 (m, Leu α-CH), 4.02 (m, Val α-CH), 4.45 (m, α-CH), 4.73 (m, α-CH), 7.09 (dd, J = 1.6, 8.7 Hz, indole H_5), 7.11 (br s, $CONH_2$), 7.13 (d, J = 2.7 Hz, indole H_2), 7.54 (d, J = 1.6 Hz, indole H_7), 7.56 (d, J = 8.7 Hz, indole H_4), 7.80 (d, J = 8.3 Hz, NH), 7.92 (br s, $CONH_2$), 8.31 (s, PhH), 10.92 (br s, indole H_1).

Isolation with Acetic-d_6 Anhydride. A small-scale isolation procedure (40 g, wet weight of sponge) employing acetic-d_6 anhydride (10 mL, 99+ atom %; Aldrich) as the acetylating agent yielded hexaacetylcelenamide-d_{18} A (5, 12 mg) and hexaacetylcelenamide-d_{18} B (6, 8 mg) which proved to be identical with 3 and 4 (TLC, IR, ^{1}H NMR), respectively, with the exception of the absence of signals in the ^{1}H NMR spectra corresponding to phenol acetate and acetamide.

Acknowledgment. We thank Michael LeBlanc (British Columbia collections) and Dr. D. J. Faulkner (La Jolla collections) for assistance in collecting *Cliona celata*. This work was supported by the Natural Science and Engineering Research Council of Canada. R.J.S. thanks the Natural Science and Engineering Research Council of Canada for a postgraduate scholarship.

Registry No. 3, 74144-16-4; 4, 74144-17-5; 5, 74144-18-6; 6, 74144-19-7; 9, 68857-44-3; 11, 74144-20-0; 12, 74144-21-1; 13, 74144-22-2; 14, 74144-23-3; 15, 74144-24-4; 16, 74144-25-5; 17, 74144-26-6; 18, 74144-27-7; 3,4,5-triacetoxybenzaldlehyde, 71932-18-8; 3,4-diacetoxybenzaldehyde, 67727-64-4; leucine, 61-90-5; valine, 72-18-4; dimethyl oxalate, 553-90-2.

Synthesis of Phosphines Having Chiral Organic Groups Ligated to Chiral Phosphorus

Donald Valentine, Jr.,* John F. Blount, and Katherine Toth

Chemical Research Department, Hoffmann-La Roche, Inc., Nutley, New Jersey 07110

Received February 1, 1980

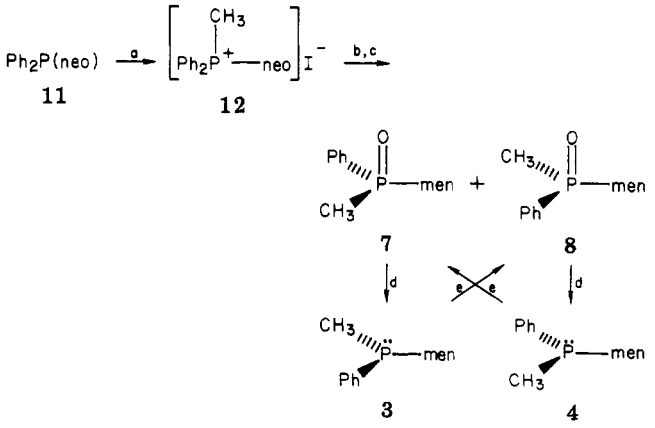
Both *R* and *S* phosphorus epimers of menthylmethylphenylphosphine and its phosphine oxide and of neomenthylmethylphenylphosphine and its phosphine oxide were prepared. The menthylmethylphenylphosphine oxides were prepared from neomenthyldiphenylphosphine by a method which is potentially general for synthesis of phosphines and phosphine oxides having chiral organic groups ligated to chiral phosphorus. Thus, neomenthyldiphenylphosphine was quaternized by methyl iodide to give neomenthylmethyldiphenylphosphonium iodide which was decomposed in boiling aqueous methanolic sodium hydroxide to give a 1:1 mixture of *R* and *S* phosphorus epimers of menthylmethylphenylphosphine oxides. The pure diastereomers were obtained from the mixture by fractional crystallization and reduced to the phosphines by using hexachlorodisilane. Structures of the *R* phosphorus epimers of both menthyl- and neomenthylmethylphenylphosphine oxides are reported. The structure of η^4-(1,5-cyclooctadiene)bis[(*R*)-menthylmethylphenylphosphine]rhodium(I) tetrafluoroborate is also reported.

Chiral phosphines have been widely used to prepare low-valent transition-metal-complex catalysts for enantioselective organic transformations.[1,2] The most commonly used type of chiral phosphine is the chelating diphosphine in which two achiral phosphorus centers are connected by a chiral link, e.g., DIOP (1),[3] or in which two chiral phosphorus centers are linked by an achiral connector, as in DIPAMP (2)[4] (Chart I). Another possibility is ligation of chiral organic groups to chiral phosphorus. Few examples are known of this type, apparently because of a lack of adequate synthetic methods. One exception is the recent description by Fisher and Mosher[5] of the diastereomeric menthylmethylphenylphosphines 3 and 4, which they prepared by condensation of sodium methylphenylphosphide with neomenthylchloride as shown in eq 1. We were also interested in these phosphines for the

$$NaP(Ph)(CH_3) + Cl(neo) \rightarrow 3 + 4 \qquad (1)$$

formation of catalysts for asymmetric hydrogenations of acrylic acids.[6] Phosphines 3 and 4 have been prepared by a convenient and potentially general new method. We have also prepared for the first time the corresponding neomenthylmethylphenylphosphines 5 and 6. This paper

Scheme I. Menthylmethylphenylphosphines and Their Phosphine Oxides[a]



[a] Conditions: a, CH_3I, 23 °C, 18 h, then reflux 2 h (95% yield); b, reflux, NaOH/aqueous CH_3OH, 18 h (88%, 7/8 ratio ~1:1); c, fractional crystallization, see text; d, Si_2Cl_6, 80 °C, 10 min, C_6H_6; e, 30% H_2O_2, 23 °C, 18 h, C_6H_6.

* To whom correspondence should be addressed at Catalytica Associates, Inc., 3255 Scott Boulevard, Suite 7E, Santa Clara, CA 95051.

0022-3263/80/1945-3691$01.00/0