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Total Syntheses with Tricyclooctanone Building Blocks. Synthesis of Iridodial

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A total synthesis of the monoterpene iridodial, in 12 steps and 13% overall yield from 1,3-cyclohexadiene, is described. The known (−)-tricyclo[3.3.0.0^{2,8}]octan-3-one, which is readily available in multigram batches and in high enantiomeric purity, served as the key intermediate. The subsequent transformations involved standard procedures, and separations were only necessary in the two final steps.

A preceding contribution[1] has dealt with the transformation of the intermediate (−)-**2**, obtained by regio- and stereoselective methylation[2] of tricyclo[3.3.0.0^{2,8}]octan-3-one [(−)-**1**][3,4] into loganin aglucon 6-acetate. It required, in particular, an oxidative enlargement and other structural modifications of ring A, which were initiated by the isomerization of the cyclopropyl ketone moiety into the 7,8-unsaturated ketone. The starting material, (−)-**1**, and its enantiomer are available in multigram batches, *each* with >98% enantiomeric excess and in ca. 17% overall yield[1,5] from 1,3-cyclohexadiene via a photochemical oxadi-π-methane rearrangement as the key step.[3,4]

We now report the first selective total synthesis of iridodial [(+)-**8**].[6] The compound is found as a constituent of ants[7] and other insects,[8] and it has been shown also to be an intermediate on the biosynthetic pathway to iridoid glucosides in plants.[9-11] The synthesis includes (−)-**1** as a key intermediate, and it is a further illustration of the potential and versatility of the optically active tricyclooctanones as building blocks in the synthesis of cyclopentanoid natural products. It documents, together with the preceding work,[1] our claim that **1** provides for a unified synthetic approach to most iridoids.[4]

For the synthesis of (+)-**8**, the strategy, which was employed in the transformation of (−)-**2**, was different from that followed in the loganin synthesis.[1] Ring B was now to be enlarged oxidatively, coupled with the introduction of a second methyl group. The task was achieved by standard procedures and a minimum set of functional group transformations. Separations could be avoided in the first five of the steps shown in Scheme I. The synthesis of (+)-**8** was completed in seven steps from (−)-**1** and 49% overall yield.[5]

Results and Discussion

Reduction of (−)-**2**[1] with DIBAH afforded 91% of the endo-alcohol (−)-**3** and merely 4% of the exo isomer (Scheme I). Reductions with lithium aluminum hydride and sodium borohydride were much less stereoselective and gave two isomers in close to equal amounts. The alcohol (−)-**3** was then treated with methanesulfonyl

(1) Demuth, M.; Chandrasekhar, S.; Schaffner, K. *J. Am. Chem. Soc.* **1984**, *106*, 1092.

(2) (a) Demuth, M.; Chandrasekhar, S.; Nakano, K.; Raghavan, P. R.; Schaffner, K. *Helv. Chim. Acta* **1980**, *63*, 2440. (b) See also: Demuth, M.; Raghavan, P. R.; Schaffner, K. *Abstr. ESOC I Conf.* **1979**, 312. Schaffner, K.; Demuth, M. *Chimia* **1981**, *35*, 437.

(3) Demuth, M.; Raghavan, P. R.; Carter, C.; Nakano, K.; Schaffner, K. *Helv. Chim. Acta* **1980**, *63*, 2434.

(4) For a review on the preparation of the tricyclooctanone enantiomers and their use in total syntheses of cyclopentanoids and related products, see: Demuth, M.; Schaffner, K. *Angew. Chem.* **1982**, *94*, 809; *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 820. For more recent work, see: Demuth, M.; Canovas, A.; Weigt, E.; Krüger, C.; Tsay, Y.-H. *Angew, Chem.* **1983**, *95*, 747; *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 721; *Angew. Chem., Suppl.* **1983**, 1053. Mikhail, G.; Demuth, M. *Helv. Chim. Acta* **1983**, *66*, 2362.

(5) All yields refer to isolated products and are corrected for purity as determined by gas chromatography.

(6) A previous synthesis, albeit of a poorly defined diastereomeric mixture of iridodials, from (−)-citronellal has been reported: Clark, K. J.; Fray, G. I.; Jaeger, R. H.; Robinson, R. *Tetrahedron* **1959**, *6*, 217. The transformation of the starting material, which is not readily available, was achieved in three steps and an overall yield of 7%. Since citronellal has been totally synthesized, this work must be considered the first total—though rather unselective—synthesis of (+)-**8**.

(7) (a) Cavill, G. W. K.; Ford, D. L.; Locksley, H. D. *Aust. J. Chem.* **1956**, *9*, 288. (b) Trave, R.; Pavan, M. *Chim. Ind. (Milan)* **1956**, *56*, 1015. (c) Cavill, G. W. K.; Ford, D. L. *Aust J. Chem.* **1960**, *13*, 296.

(8) Abou-Donia, S. A.; Fish, L. J.; Pattenden, G. *Tetrahedron Lett.* **1971**, 4037.

(9) Inouye, K.; Ueda, S.-I.; Uesato, S.-I.; Kobayashi, K. *Chem. Pharm. Bull.* **1978**, *26*, 3384.

(10) See El-Naggar, L. J.; Beal, J. L. *J. Nat. Prod.* **1980**, *43*, 649, for references concerning the potential pharmaceutical significance of the iridoids.

(11) The structure of iridodial has not been established in all details. For a review of the early structural studies, see: Cavill, G. W. K. In "Insect Terpenoids and Nepetalactone"; Taylor, W. I., Battersby, A. R., Eds.; Marcel Dekker: New York, 1969; p 203. Cavill[7c] and Inouye[9] claim that naturally occurring materials possess tautomeric enol hemiacetal structures and undergo some undefined changes on steam distillation.

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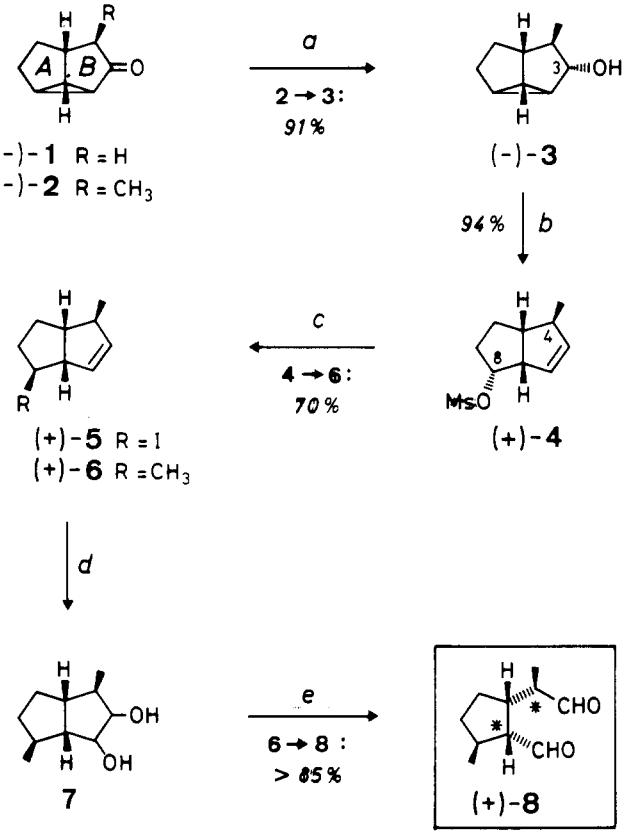
Scheme I. Synthesis of Iridodial [(+)-8]

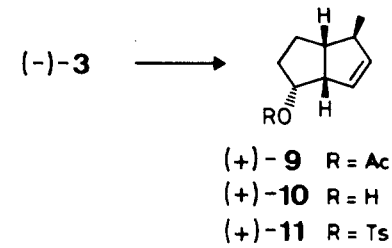


[a] DIBAH, ether, −78 °C [(−)-2 → (−)-3]. [b] Methanesulfonyl chloride, triethylamine, 0 °C. [c] NaI, acetone, reflux [(+)-4 → (+)-5]; CH_3MgI, CuI, ether, room temperature [(+)-5 → (+)-6]. [d] Catalytic amount of OsO_4, *N*-methylmorpholine *N*-oxide, aqueous *tert*-butyl alcohol-acetone, room temperature. [e] $NaIO_4$, ether-H_2O (1:1), 5 °C.

chloride and triethylamine at 0 °C, which afforded directly the unsaturated endo-mesylate (+)-4 as the main component of a 5:1 mixture of C(8) epimers. The 8-endo configuration of the main component was assigned upon comparison of the ^1H NMR data with those of a sterically uniform 8-endo mesylate prepared separately from (+)-10 by the route shown in Scheme II. The alcohol (+)-10 was obtained in an α-hydroxycyclopropane rearrangement of (−)-3 under reaction conditions known from analogous examples in steroid chemistry.[12] Treatment of (−)-3 with acetic anhydride and boron trifluoride gave the rearranged 8-endo-acetate (+)-9, which was then hydrolyzed to the alcohol (+)-10.

The mixture of epimeric mesylates 4 (Scheme I) was then treated with sodium iodide. The substitution of the sulfonyloxy group proceeded with 4.5:1 stereoselection in favor of the inversion to the 8-exo product (+)-5. When the mixture of epimeric iodides was treated with methyl Grignard reagent in the presence of cuprous iodide the exo isomer selectively reacted with retention of configuration to give the exo-alkylated product, (+)-6, in high yield. The minor isomer, 8-*endo*-5, was much less reactive and, on prolonged reaction time, underwent elimination to the 7,8-olefin. Experiments with other coupling reagents were less successful. The methyl Grignard reagent and dimethyl cuprate variants with the acetate (+)-9 and the *p*-toluenesulfonate (+)-11 (Scheme II) either afforded predominantly elimination or did not react at all.[13]

Scheme II[a]



[a] (−)-3 → (+)-9: Ac_2O, BF_3 etherate, 0 °C. (+)-9 → (+)-10: KOH, CH_3OH, room temperature. (+)-10 → (+)-11: *p*-toluenesulfonyl chloride, pyridine, 5 °C.

The hydrocarbon (+)-6 is the first product that was purified prior to continuing in the synthetic sequence. With this compound the basic carbon skeleton, containing four adjacent asymmetric carbon atoms and the functionality required for the ultimate transformation to the target structure, was completed. Vicinal hydroxylation of the double bond was almost quantitative when carried out with a catalytic amount of osmium tetroxide in combination with *N*-methylmorpholine *N*-oxide.[14] The reaction afforded a 3:1 mixture of the epimeric diols (7, Scheme I). Cleavage of the mixture with sodium periodate finally gave iridodial, [(+)-8]. The product was isomerically pure by gas chromatography and high-resolution ^1H NMR spectroscopy, a criterium that has not been met by earlier preparations.[6,9,15] The ^1H NMR and IR spectra of freshly prepared material clearly showed that the dialdehyde was present both in carbon tetrachloride and in chloroform, and they gave no indication of the tautomeric enol hemiacetal structure that natural iridodial samples have been found to possess.[7c,9,11]

In accord with the literature, the dialdehyde (+)-8 showed a tendency to polymerize, and the monomer could be recovered upon distillation.[7a] Although the IR and mass spectral data of the synthetic (+)-8 were in satisfactory accord with those reported for iridodial isolated from natural sources,[7,9,16] distillation was found to cause partial epimerization, as became clearly evident from ^1H NMR spectroscopy. Thus, the doublets for the two secondary methyl groups at δ 1.10 and 1.14 were substituted by a more complex signal pattern in the range of δ 1.0–1.15, and the doublets for the two aldehydic protons at δ 9.61 and 9.70 were now accompanied by two additional such doublets at δ 9.63 and 9.74. Either or both starred methines of formula (+)-8 are possible sites for this change.

The literature values for rotations had all been determined with iridodial samples that had been distilled prior to the measurements and that consequently were not isomerically uniform. For freshly synthesized iridodial (8), rotations ($[\alpha]^{23}_D$) of +38.1° in methanol and +34° in chloroform were measured. When during the distillation of this material various fractions were collected, the individual rotations were higher than before, as were also

(12) Narayanan, C. R.; Prakash, S. R.; Nagasampagi, B. A. *Chem. Ind.* **1974**, 966.

(13) For the successful coupling of the methyl Grignard reagent with several examples of *p*-toluenesulfonates and with an allylic acetate, see: Fouquet, G.; Schlosser, M. *Angew. Chem.* **1974**, *86*, 50; *Angew. Chem., Int. Ed. Engl.* **1974**, *13*, 82.

(14) VanRheenen, V., Kelly, R. C.; Cha, D. Y. *Tetrahedron Lett.* **1976**, 1973.

(15) The configuration shown for the starred asymmetric carbon atoms of (+)-8 follows unequivocally from the precursors of the synthetic compound, but it has, in fact, not been established for the natural material.[11]

(16) Vidari, G.; De Bernardi, M.; Pavan, M.; Ragozzino, M. *Tetrahedron Lett.* **1973**, 4065.

(17) See ref 1 for the analytical data of (−)-2, except for the ^1H NMR spectrum, which is given by: Demuth, M.; Mikhail, G. *Tetrahedron* **1983**, *39*, 991.

the literature values ($[\alpha]^{17}_D$ +44.5°[7b] and $[\alpha]^{20}_D$ +67.7°,[9] both in methanol), and they even varied over a wider range (from +38.1 to +71.5° in methanol).

With the accomplished synthesis structurally well-defined material is now accessible that may help to elucidate the stereochemistry and other unresolved structural aspects of the natural iridodials.[11]

Experimental Section

Melting points were taken under a microscope on a Kofler hot plate and are uncorrected. Specific optical rotations, $[\alpha]_D$, were measured at 23 °C in $CHCl_3$, *c* in parentheses, experimental error ±5%. ^1H NMR spectra were run in $CDCl_3$, unless stated otherwise, on Bruker WP-80 and WH-270 instruments in FT mode at 80 and 270 MHz, respectively. The chemical shifts are in parts per million [δ values with $(CH_3)_4Si$ as internal reference], and the coupling constants (*J*) are in hertz. Abbreviations used are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. IR spectra were run in $CHCl_3$, unless stated otherwise, on Perkin-Elmer 137 and 700 instruments and are given in reciprocal centimeters. Mass spectra (MS; in *m/e*) were recorded on a Varian MAT CH5 instrument at 70 eV. Elemental analyses were performed by Dornis and Kolbe, Mülheim a.d. Ruhr. Gas chromatographic (GLC) analyses were carried out with a Varian Aerograph 1700 instrument equipped with a flame-ionization detector coupled to a Spectra Physics Autolab System I computing integrator. OV 101 glass capillary columns of 20- and 35-m length were used, with N_2 as the carrier gas. The solvents were purified by standard procedures. All reactions were run under Ar atmosphere. In the normal workup operation, the solvent was removed in vacuo, and the residue was taken up in ether and water; after shaking, the organic layer was separated, dried over $MgSO_4$, and filtered. The solvent of the filtrate was removed by distillation. The analytical samples of all new compounds were purified by column chromatography on silica gel (Merck; 0.043–0.06 mm), unless stated otherwise. The purity was >96% by GLC in all cases.

(−)-3-*endo*-Hydroxy-4-*exo*-methyltricyclo[3.3.0.0^{2,8}]octane (3). A 1 M solution of DIBAH in *n*-hexane (13 mL) was added dropwise to a stirred solution of (−)-**2**[17] (1.73 g; purity 96% by GLC; 12.1 mmol) in ether (40 mL) at −78 °C. The reaction was quenched after 6 h by the addition of aqueous HCl (10%, 3 mL) and warming up to room temperature. Brine was then added, and the aqueous layer was saturated with NaCl and extracted several times with ether. The combined organic portions were dried over $MgSO_4$, and the solvent was removed by vacuum distillation to afford 1.70 g of (−)-**3** (purity 90% by GLC; 91% yield). A sample was chromatographed on silica gel (1:50; ether–pentane, 1:1): $[\alpha]_D$ −40° (*c* 0.38); ^1H NMR δ 0.87 (3 H, d, *J* = 8 Hz), 1.0–2.35 (10 H, 1 H exchangeable with D_2O, m) 4.02 (1 H, d, *J* = 6 Hz); IR 3600, 3440, 1600, 1060, 1015, 975, 945 cm^{-1}; MS, *m/e* 138 (M^+), 120, 109, 94, 80, 67 (base peak), 57, 41. Anal. Calcd for $C_9H_{14}O$: C, 78.26; H, 10.14. Found: C, 78.28; H, 10.16.

8-*endo*- and 8-*exo*-4-*exo*-Methylbicyclo[3.3.0]oct-2-en-8-yl *p*-Methanesulfonates 4. (−)-**3** (0.92 g; purity 90% by GLC; 6 mmol) was dissolved in dichloromethane (15 mL). To the stirred solution at 0 °C were then added methanesulfonyl chloride (0.8 g, 7 mmol) and triethylamine (0.8 mL). After a reaction time of 1 h, the mixture was poured into chilled H_2O. The organic layer was separated and consecutively washed with ice-cold 10% HCl and $NaHCO_3$ solutions. After drying of the organic portions over $MgSO_4$, the solvent was removed in vacuo, affording a 5:1 mixture of 8-*endo*- and 8-*exo*-**4** as a colorless oil (1.37 g; purity 89% by GLC; 94% yield), which was used further without purification. Data of the crude mixture: ^1H NMR δ 0.97 (3 H, d, *J* = 8 Hz), 1.2–2.3 (4 H, m), 2.4 (2 H, m), 2.98 (3 H, s), 3.4 (1 H, m), 4.65 + 4.7 (ratio 5:1, 1 H, each m), 4.85 + 4.96 (ratio 5:1, 1 H, each s), 5.50 + 5.55 (ratio 5:1, 1 H, each m); IR 1620, 1460, 1360, 1340, 1180, 920 cm^{-1}; MS, *m/e* 216 (M^+, $C_{10}H_{16}O_3S$), 135, 120 (base peak), 79, 55, 41.

A sterically uniform sample of **(+)-4-*exo*-methylbicyclo[3.3.0]oct-2-en-8-*endo*-yl *p*-methanesulfonate (4)** was prepared for comparison purposes from (+)-**10** following the above procedure: $[\alpha]_D$ +75° (*c* 0.05). For the ^1H NMR data, see the data above of the major stereoisomer. The IR and MS spectra were indistinguishable from those of the mixture.

(+)-4,8-*exo*-Dimethylbicyclo[3.3.0]oct-2-ene (6). NaI (1.5 g, 15 mmol) was added to an acetone solution (20 mL) of the mixture of epimeric mesylates **4** (0.82 g; 89% purity by GLC; 3.4 mmol), which was then refluxed for 4 days. The workup gave 0.7 g of a 4.5:1 mixture of **4-*exo*-methyl-8-*exo*-** and **4-*exo*-methyl-8-*endo*-iodobicyclo[3.3.0]oct-2-enes (5),** which were used further without purification. Data of the crude mixture: $[\alpha]_D$ +70° (0.05); ^1H NMR δ 1.03 + 1.11 (ratio 4.5:1, 3 H, each d, *J* = 7 Hz), 1.6–1.75 (2 H, m), ca. 1.9 + 2.15 + 3.12 + 5.55 + 5.58 (each 1 H, m), 2.25–2.5 (2 H, m), 4.87 + 4.98 (ratio 4.5:1, 1 H, each m); IR 1610, 1215, 1095, 1030 cm^{-1}; MS, *m/e* 248 (M^+, $C_9H_{13}I$), 121 (base peak), 93, 79, 55.

Methylmagnesium Grignard reagent (0.5 g of Mg = 20.8 mmol, 1.5 mL of CH_3I = 24 mmol) was prepared in ether (80 mL) at reflux temperature. After the solution was cooled to 0 °C, CuI (1.6 g, 8.4 mmol) was added in portions. Rewarming to room temperature was followed by stirring for 15 min and subsequent addition of (+)-**5** (0.7 g in 10 mL of ether). After 24 h of stirring, the mixture was worked up, and the solvent was carefully distilled off in vacuo. Chromatography on silica gel (1:50; ether–pentane, 1:3) afforded (+)-**6** (0.24 g, 70% yield): $[\alpha]_D$ +156° (*c* 0.05); ^1H NMR δ 1.00 (3 H, d, *J* = 8 Hz), 1.10 (3 H, d, *J* = 9 Hz), 1.5–2.6 (8 H, m), 5.50 + 5.60 (each 1 H, d, *J* = 7 Hz); MS, *m/e* 136 (M^+, $C_{10}H_{16}$), 121, 105, 93, 79, (base peak), 41.

Iridodial [(+)-8]. (+)-**6** (0.15 g, 1.1 mmol) was hydroxylated with a catalytic amount of OsO_4 in combination with *N*-methylmorpholine *N*-oxide in *tert*-butyl alcohol–acetone–H_2O at room temperature.[14] A mixture (0.18 g) of epimeric **4,8-*exo*-dimethylbicyclo[3.3.3]octane-2,3-diols (7)** was obtained. Data of **7** (1:3 mixture by GLC): ^1H NMR δ 0.96 + 1.04 (ratio 1:3, 6 H, each d, *J* = 6 Hz), 1.15–2.1 (7 H, m), ca. 2.3 (1 H, m), ca. 2.0 + ca. 2.6 (each 1 H, broad, exchangeable with D_2O), ca. 3.7 + 3.85 (each 1 H, m); IR 3400, 1450, 1090 cm^{-1}; MS, *m/e* 170 (M^+), 152, 137, 123, 109, 94, 81 (base peak), 71, 55, 41, 27. Anal. Calcd for $C_{10}H_{18}O_2$: C, 70.56; H, 10.59. Found: C, 70.66; H, 10.72.

The crude mixture of **7** (0.12 g, 0.7 mmol) was dissolved in ether (10 mL), aqueous $NaIO_4$ (0.2 g in 10 mL of H_2O) was added, and the solution was stirred for 24 h at 0–5 °C. The workup gave 0.108 g of isomerically pure (+)-**8** (purity >95% by GLC; 87% yield). Data of freshly synthesized nondistilled compound: $[\alpha]_D$ +34° (*c* 0.1, $CHCl_3$), +38.1° (*c* 0.02, CH_3OH); the concentration dependence of $[\alpha]_D$ was negligible at these dilutions; ^1H NMR δ 1.10 + 1.14 (each 3 H, d, *J* = 6 Hz), 1.2–1.7 (4 H, m), 1.95 (2 H, m), 2.38 + 2.74 (each 1 H, m), 9.61 (1 H, d, *J* = 2 Hz), 9.70 (1 H, d, *J* = 1.5 Hz); IR (CCl_4) 2710, 1720, 1460, 1375 cm^{-1}; MS, *m/e* 168 (M^+, $C_{10}H_{16}O_2$), 153, 139, 111, 81, 67, 41 (base peak).

Data of a sample distilled at 10^{-2} torr: the $[\alpha]_D$ values of individual fractions collected at 50–60 °C ranged from +38.1 to +71.5° (*c* 0.02, CH_3OH); ^1H NMR δ 1.0–1.15 (6 H, complex), 1.2–1.7 (4 H, m), 1.95 (2 H, m), ca. 2.4 + 2.75 (each 1 H, m), 9.61 (1 H, d, *J* = 2 Hz), 9.63 (1 H, d, *J* = 1 Hz), 9.70 + 9.74 (each 1 H, d, *J* = 1.5 Hz); the integration of the individual aldehydic proton signals varied with the distillation fractions; IR (CCl_4) and MS as above. For comparison with literature data, see text and ref 7b, 9, 16.

(+)-4-*exo*-Methylbicyclo[3.3.0]oct-2-en-8-*endo*-yl Acetate (9). (−)-**3** (0.46 g; purity 90% by GLC; 3 mmol) was treated at 0 °C with acetic anhydride (20 mL) and BF_3 etherate (2 mL). After 15 min the mixture was poured into chilled H_2O and neutralized with Na_2CO_3. The workup gave 0.51 g of (+)-**9** (purity 85% by GLC; 81% yield). A sample was chromatographed on silica gel (1:50; ether–pentane, 1:3): $[\alpha]_D$ +186° (*c* 0.37); ^1H NMR δ 1.01 (3 H, d, *J* = 8 Hz), 1.47 + 1.98 + 3.12 + 4.86 (each 1 H, m), 1.66 + 2.33 (each 2 H, m), 2.01 (3 H, s), 5.52 + 5.57 (each 1 H, d, *J* = 6 Hz); IR 1720, 1600, 1230, 1025. MS, *m/e* 180 (M^+, $C_{11}H_{16}O_2$), 120, 105, 55, 43 (base peak).

(+)-4-*exo*-Methylbicyclo[3.3.0]oct-2-en-8-*endo*-ol (10). (+)-**9** (0.97 g; purity 85% by GLC; 4.6 mmol) was dissolved in CH_3OH (20 mL), and a KOH solution (2 g in 20 mL of H_2O) was added. After the solution was stirred at 60 °C for 15 min, the workup gave 0.74 g of (+)-**10** (purity 82% by GLC; 98% yield). A sample was chromatographed on silica gel (1:40; ether–pentane, 1:1): $[\alpha]_D$ +180° (*c* 0.45); ^1H NMR δ 1.01 (3 H, d, *J* = 8 Hz), 1.2 – 2.2 (5 H, 1 H exchangeable with D_2O, m), 2.32 (2 H, m), 3.05 (1 H, m), 4.01 (1 H, s), 5.48 + 5.55 (each 1 H, d, *J* = 6 Hz); IR 3600, 3440,

1600, 1060, 1015, 975, 945; MS, m/e 138 (M^+), 120, 105, 94, 81, 79 (base peak), 67. Anal. Calcd for $C_9H_{14}O$: C, 78.26; H, 10.14. Found: C, 78.09; H, 9.95.

(+)-4-*exo*-Methylbicyclo[3.3.0]oct-2-en-8-*endo*-yl *p*-Toluenesulfonate (11). A solution of (+)-**10** (0.61 g; purity 82% by GLC; 3.6 mmol) and *p*-toluenesulfonyl chloride (0.9 g, 4.7 mmol) in pyridine (15 mL) was kept at 5 °C for 16 h. Extraction with CH_2Cl_2 and H_2O (2 times) and with cold aqueous 10% HCl (3 times), drying of the organic layer with $MgSO_4$, and evaporation of the solvent gave 1.05 g of (+)-**11** (purity 81% by GLC; 85% yield). A sample was chromatographed on Florisil (60–100 mesh; 1:40; ether–pentane, 1:1): $[\alpha]_D$ +38° (c 0.2); ^{1}H NMR δ 0.92 (3 H, d, J = 8 Hz), 1.35 + 1.55 + 1.67 + ca. 1.9 (each 1 H ,m), 2.27 (2 H, m), 2.37 (3 H, s), ca. 3.2 (1 H, m), 4.56 (1 H, s), 5.22 + 5.50 (each 1 H, d, J = 6 Hz), 7.25 + 7.71 (each 2 H, d, J = 8 Hz); IR 1600, 1450, 1355, 910; MS, m/e 120 [M^+ ($C_{16}H_{20}SO_3$) – $C_7H_7SO_3H$; base peak], 105, 93, 77.

Registry No. (–)-**2**, 88195-49-7; (–)-**3**, 88915-72-4; (+)-**4**, 88915-73-5; 8-*exo*-**4**, 88979-72-0; (+)-**5**, 88915-74-6; (+)-**6**, 88915-75-7; **7**, 88915-76-8; (+)-**8**, 88979-71-9; (+)-**9**, 88915-77-9; (+)-**10**, 88915-78-0; (+)-**11**, 88915-79-1.

Synthesis of Peptide Analogues Containing (2-Aminoethyl)phosphonic Acid (Ciliatine)[1]

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Di- and tripeptide analogues containing (2-aminoethyl)phosphonic acid [H–Aep(OH)$_2$], which has been discovered in a wide variety of living organisms, were prepared. As starting materials for incorporating the amino phosphonic acid into a peptide chain, the N-phthalylated diethyl ester [Pht–Aep(OEt)$_2$] and the N-carbobenzyloxylated monoalkyl ester [Cbz–Aep(OR)(OH), R = Me, Et, and Bzl] were used. A phosphonamide bond between the amino phosphonic acid unit and amino acid unit was formed by reaction of Pht–Aep(OEt)Cl with amino acid ethyl ester or coupling reaction between Cbz–Aep(OR)(OH) and amino acid ethyl ester using diphenylphosphoryl azide–triethylamine as a condensing agent. Removal of the protecting groups was studied in connection with the acid-labile phosphonamide bond in the abnormal peptides.

Introduction

Amino phosphonic acids, H_2H–R–PO_3H_2, may be considered to be analogues of amino carboxylic acids and amino sulfonic acids. (2-Aminoethyl)phosphonic acid (ciliatine) [1, H–Aep(OH)$_2$] would be the most interesting compound of the class and was discovered in a wide variety of living organisms ranging from lower protozoans to higher animals including man. Many reports have been concerned with the involvement of **1** in phosphonolipids,[2] while some have shown the presence of **1** in the proteinaceous fractions of sea anemones *Metridium dianthus*[3,4] and *Metridium senile*,[5] the protozoan *Tetrahymena pyriformis*,[6] as well as Chymotrypsin-like proteases from *M. senile*,[7] though the binding mode in the proteins has not been investigated thoroughly.[8]

Parallel with these studies, the synthesis of peptides containing amino phosphonic acids was carried out.[9-17] Biological activities of the artificial peptides appeared to be promising. For instance, L-alanyl-L-(1-aminoethyl)-phosphonic acid was shown to exhibit a very strong antibacterial activity, especially against Gram-negative organisms,[15] and *N*-[[[(carbobenzyloxy)amino]methyl]-hydroxylphosphinyl]-L-phenylalanine was found to function as a potent inhibitor against carboxypeptidase A.[16] Compound **1** was also reported to inhibit bacterial cell wall synthesis.[18]

The synthetic studies, however, dealt exclusively with unnatural (aminomethyl)phosphonic acids and their α-

(1) Abbreviations: Bzl = benzyl; Cbz = carbobenzyloxy; Np = p-nitrophenyl; Pht = phthalyl; H–(aa)–OH = amino acid; DMF = dimethylformamide; THF = tetrahydrofuran; TLC = thin layer chromatography. Derivatives of (2-aminoethyl)phosphonic acid are expressed as the following examples. $C_6H_5CH_2O_2CNHCH_2CH_2P(O)(OR_1)(OR_2)$ = Cbz–Aep(OR$_1$)(OR$_2$); $H_2NCH_2(O)NHCH_2CH_2P(O)(OC_2H_5)NHCH_2CO_2Li$ = H–Gly–Aep(OEt)–Gly(OLi).

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(8) The amino phosphonic acid content of the proteins varied from sample to sample. Some had significantly high value in comparison to the number of total amino acid residues. These, together with various chemical tests, lend support to the hypothesis that **1** may be bound directly to the protein as constituents within the peptide backbone, directly to the side chains of the amino acid residues such as lysine, or indirectly to the protein via a lipid or a polysaccaride bridge (ref 3–7).

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