Pyrrolizidine Alkaloid Analogues. Synthesis of 11-Membered Macrocyclic Diesters of (+)-Heliotridine

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The first synthesis of macrocyclic diesters incorporating (+)-heliotridine has been achieved. Treatment of (+)-heliotridine (**1**) with different glutaric anhydride derivatives produced mainly the corresponding 9-monoesters of heliotridine. Lactonisation was carried out after formation of the pyridine-2-thiol esters to give a range of 11-membered macrocyclic diesters [(**4**)—(**8**)] of heliotridine. The structures of these new pyrrolizidine alkaloid analogues were established by comparison of their ^{1}H n.m.r. and mass spectra with those of natural macrocyclic pyrrolizidine alkaloids. Attempts to make 10-membered macrocyclic diesters of heliotridine were unsuccessful.

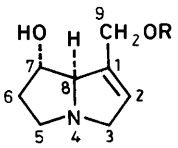
Many pyrrolizidine alkaloids occur as macrocyclic diesters with a pyrrolizidine diol or triol in combination with a diacid moiety.[1,2] The majority of these dilactones contain (+)-retronecine (**2**) as the base portion and are hepatotoxic. The structural requirements for toxicity are a pyrrolizidine nucleus combined with an allylic ester function [as in ester (**3**)].[3] The toxic action is believed to involve oxidation of the 2,5-dihydropyrrole system to the corresponding pyrrole by liver oxidase enzymes. These pyrrole metabolites can then act as alkylating agents by displacement of the ester group assisted by the pyrrole system. The most toxic alkaloids and analogues are macrocyclic diesters of (+)-retronecine (**2**) which can act as bifunctional alkylating agents involving removal of both ester groups. Synthetic routes to macrocyclic alkaloids and structurally related analogues are required for the study of the structure–activity relationships in this area. Synthesis of macrocyclic pyrrolizidine alkaloids has been restricted to a few containing (+)-retronecine, namely (+)-dicrotaline,[4] (±)-fulvine,[5] (±)-crispatine,[5] integerrimine,[6] and the *O*-acetyl derivative of crobarbatine.[7] Some 11-membered[8] and 10-membered[9] analogues containing (+)-retronecine have also been prepared.

Although a large number of monoester and diester derivatives of (+)-heliotridine (**1**) have been isolated,[1] it is curious that no macrocyclic diesters of this base have so far been found. We therefore decided to discover if macrocyclic diesters of (+)-heliotridine could be prepared, so that their toxicity could be evaluated.

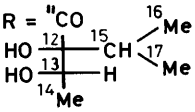
Results and Discussion

Cynoglossum officinale is known as the hound's tongue from the shape of its leaves. It is reported to contain a mixture of pyrrolizidine alkaloids, which are all monoesters or diesters of (+)-heliotridine (**1**).[1] A cultivar of this species produced echinatine (**3**) as the major (>95%) alkaloidal constituent. (+)-Echinatine was identified by comparison of its spectroscopic properties and rotation with literature values.[10] Alkaline hydrolysis of echinatine (**3**), or the total alkaloid mixture yielded (+)-heliotridine (**1**).

Treatment of (+)-heliotridine (**1**) with 3,3-dimethylglutaric anhydride in dry 1,2-dimethoxyethane (DME) gave a quantitative yield of monoesters of (+)-heliotridine. Diester formation was not observed, probably because the initial monoester products are zwitterionic and precipitate from DME. Indeed, the completeness of this step is most conveniently monitored on t.l.c. by the disappearance of (+)-heliotridine. The ^{1}H n.m.r. spectrum of the precipitate in deuteriomethanol showed signals



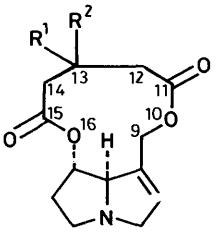
(**1**) R = H
(**2**) R = H, 7β-OH
(**3**) R = "CO



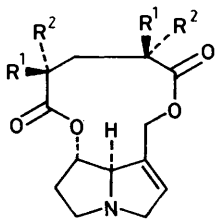
for the 9-monoester at δ 4.26 (7-H), 4.40 and 4.71 (2 H, AB system, 9-H_2), and 5.53 (2-H) and for the 7-monoester at δ 5.00 (7-H), 4.31 (2 H, s, 9-H_2), and 5.50 (2-H). From the appearance and integrations of these signals[11] the ratio of 9- to 7-monoesters is 4:1. Lactonisation of the mixture of monoesters was achieved *via* the pyridine-2-thiol esters. These were prepared by addition of 2,2′-dithiodipyridine and triphenylphosphine to a suspension of the heliotridine monoesters in DME.[12] The mixture was stirred vigorously until a homogeneous solution was obtained and formation of the thiol esters was complete. Lactonisation was carried out by heating the diluted mixture at reflux in DME for 14 h. Isolation and purification of the dilactone by column chromatography on basic alumina afforded a 61% yield of the crystalline (3,3-dimethylglutaryl)heliotridine (**4**). An accurate mass measurement on the base (**4**) gave the molecular formula $C_{15}H_{21}NO_4$. Furthermore, in the mass spectrum of the dilactone (**4**), the fragmentation pattern was similar to those recorded for macrocyclic diesters of (+)-retronecine (**2**). The major fragments at *m/z* 137, 136, 120, 119, 118, and 117 arise by cleavage of the allylic ester and subsequent loss of the diacid portion. An important feature of the ^{1}H n.m.r. spectrum of the dilactone (**4**) in deuteriochloroform is an AB system at δ 4.44 and 5.05 due to the diastereotopic protons at C-9. The chemical shift difference of 0.61 p.p.m. for these C-9 protons is within the typical range observed for 11-membered macrocyclic diesters of (+)-retronecine.[1,2] The distinctive mass spectrum of the base (**4**) together with the appreciable non-equivalence of the protons at C-9 are good evidence for the formation of an 11-membered macrocyclic diester of (+)-heliotridine (**1**).

Similar treatment of (+)-heliotridine (**1**) with tetramethyleneglutaric anhydride and with pentamethyleneglutaric anhydride produced two more crystalline macrocyclic diesters (**5**) and (**6**) in 57 and 41% yields, respectively. Significant non-

equivalence of the protons at C-9 in their ^{1}H n.m.r. spectra was observed of 0.49 p.p.m. for the dilactone (**5**) and 0.74 p.p.m. for the dilactone (**6**). A much lower chemical shift difference of 0.12 p.p.m. was recorded for the analogue (**7**), which was prepared by lactonisation of (+)-heliotridine with glutaric anhydride. The dilactone (**7**) was isolated as an oil, which could not be crystallised, in 41% yield.



(**4**) $R^1 = R^2 = Me$
(**5**) $R^1, R^2 = (CH_2)_4$
(**6**) $R^1, R^2 = (CH_2)_5$
(**7**) $R^1 = R^2 = H$



(**8**) **a**; $R^1 = Me, R^2 = H$
b; $R^1 = H, R^2 = Me$

Attempts were made to prepare macrocyclic diesters of (+)-heliotridine containing 10-membered rings. Treatment of (+)-heliotridine (**1**) with succinic anhydride and formation of the pyridine-2-thiol esters proceeded normally. When these esters were heated at reflux in DME under high dilution conditions, t.l.c. data was obtained for the formation of the succinyl-heliotridine but no cyclised product could be isolated. When phthalic anhydride was used in this procedure, no evidence was obtained for the formation of a macrocyclic dilactone. Models of the 10-membered macrocyclic diesters of (+)-heliotridine indicated that there may be unfavourable steric interactions in the macrocyclic systems.

Finally, it was decided to prepare dilactones (**8**) with substituents at the α-positions of the diacid portion to create steric hindrance around these positions in the dilactones. This is expected to enhance the toxicity of these compounds by reducing their tendency to be detoxified by hydrolysis. Accordingly, reaction of *meso*-2,4-dimethylglutaric anhydride with (+)-heliotridine and subsequent lactonisation *via* the pyridine-2-thiol esters yielded a mixture of two diastereoisomers (**8**) which could not be separated. However, consideration of the ^{1}H and ^{13}C n.m.r. spectra of the mixture (**8**) indicated that the diastereoisomers are present in a ratio of 2:1, and that the chemical shift differences for the C-9 protons are 0.17 p.p.m. for the major isomer and 0.29 p.p.m. for the minor component.

The different chemical shift differences for the C-9 protons of the new pyrrolizidine alkaloid analogues [(**4**)—(**8**)] are believed to reflect the different conformations of the diacid portions in these macrocyclic systems. *X*-Ray data on 11-membered macrocyclic diesters of retronecine (**2**) have shown that most have ester carbonyl groups that are synperiplanar, while for 12 membered dilactones of retronecine, the ester carbonyl groups are antiperiplanar.[1] It is important to establish the conformations of these macrocyclic compounds because they may favour oxidation of the 2,5-dihydropyrrole rings to the toxic pyrrole metabolites rather than detoxification processes of *N*-oxidation or hydrolysis of the lactones. Attempts will be made to establish the conformations of some of these new alkaloid analogues by *X*-ray crystallography. The toxicity of these 11-membered pyrrolizidine alkaloid analogues will also be investigated.

Experimental

M.p.s were measured with a Kofler hot-stage apparatus. Organic solutions were dried with anhydrous Na_2SO_4, and solvents were evaporated off under reduced pressure below 50 °C. N.m.r. spectra were recorded for solutions in deuteriochloroform with tetramethylsilane as the internal standard on a Bruker WP-200 SY spectrometer operating at 200 MHz unless otherwise stated. Mass spectra were obtained with A.E.I. MS 12 or 902 spectrometers. Optical rotations were measured with an Optical Activity Ltd. AA-1010 Polarimeter. T.l.c. of the bases was carried out on Kieselgel G plates of 0.25-mm thickness developed with chloroform–methanol–conc. ammonia (85:14:1). The location of the bases was carried out by oxidation with *o*-chloranil, followed by treatment with Ehrlich's reagent.[13] 1,2-Dimethoxyethane (DME) was dried by distillation from potassium hydroxide and then from sodium and benzophenone under argon immediately prior to use. *Cynoglossum officinale* seeds were obtained from Suttons Seeds Ltd. and plants were grown in the open ground.

(+)-*Echinatine* (**3**).—Freshly harvested young *C. officinale* (5 kg) were soaked overnight in methanol and extracted repeatedly with methanol until the extracts were colourless. The combined methanol extracts were concentrated under reduced pressure. The residue was taken up in methylene chloride (100 ml) and extracted with 2M-sulphuric acid (2 × 100 ml). The combined acidic layers were washed with methylene dichloride (4 × 100 ml) and stirred with powdered zinc metal (10 g) for 4 h. After having been filtered through Celite 535, the solution was made alkaline with conc. ammonia and extracted with chloroform (4 × 100 ml). The aqueous solution was basified more strongly by the addition of potassium hydroxide and extracted with chloroform (4 × 100 ml). The combined chloroform extracts were dried, filtered, and concentrated to a light brown foam, which contained one major component, R_F 0.30. Purification by chromatography on basic alumina and elution with 25% v/v chloroform in dichloromethane gave echinatine (**3**) as a gum, (5.06 g, 0.1%), $[\alpha]_D^{20}$ +12.3° (*c* 0.94, $CHCl_3$) (lit.,[10] $[\alpha]_D^{22}$ + 15.0°); $\nu_{max.}$ (thin film) 3 400, 2 973, 2 936, 2 885, 1 728, and 1 230 cm^{-1}; δ_H 0.89 and 0.93 (6 H, both d, *J* 6.8 Hz, 16- and 17-H_3), 1.27 (3 H, d, *J* 6.6 Hz, 14-H_3), 1.86 (1 H, m, 6-H), 1.96 (1 H, m, 6-H), 2.18 (1 H, dq, *J* 6.8 Hz, 15-H), 2.62 (1 H, ddd, *J* 10.7, 7.0, and 6.1 Hz, 5-H), 3.27 (1 H, dd, *J* 10.8 and 6.5 Hz, 5-H), 3.37 (1 H, dd, *J* 3.0 and 1.5 Hz, 3-H), 3.60 (1 H, br s, OH), 3.88 (1 H, dd, *J* 3.1 and 1.5 Hz, 3-H), 3.97 (1 H, m, 8-H), 3.99 (1 H, q, *J* 6.6 Hz, 13-H), 4.01 (2 H, br s, 2 × OH), 4.15 (1 H, dt, *J* 6.0 Hz, 7-H), 4.79 and 4.96 (2 H, AB system, *J* 13.4 Hz, 9-H_2), and 5.70 (1 H, br s, 2-H); δ_C(50 MHz) 15.7 and 17.8 (C-16 and C-17), 17.2 (C-14), 32.2 (C-15), 33.5 (C-6), 54.2 (C-5), 61.7 (C-3), 62.0 (C-9), 71.6 (C-13), 74.2 (C-7), 79.7 (C-8), 84.1 (C-12), 125.6 (C-2), 136.1 (C-1), and 173.9 (C-11); *m/z* 299 (M^+, 4%), 156, 139, 138 (100), 137, 136, 120, and 95 (Found: M^+, 299.1735; C, 60.6; H, 8.21; N, 4.35%. $C_{15}H_{25}NO_5$ requires *M*, 299.1732; C, 60.18; H, 8.42; N, 4.68%). The *picrolonate** had m.p. 210—212 °C (lit.,[10] 214 °C) (Found: C, 53.4; H, 6.1; N, 12.6. $C_{25}H_{33}N_5O_{10}$ requires C, 53.3; H, 5.9; N, 12.4%).

(+)-*Heliotridine* (**1**).—Echinatine (1.02 g, 3.39 mmol) was heated at reflux with barium hydroxide (2.00 g, 11.67 mmol, 3.44 equiv.) in water (25 ml) for 4 h. Solid carbon dioxide was added to the cooled solution which was then filtered. The filtrate was basified to >pH 10 with potassium hydroxide and continuously extracted with chloroform for 48 h to yield (+)-heliotridine (450 mg, 85%) m.p. 116—117 °C (acetone) (lit.,[10] m.p. 115—116 °C); $[\alpha]_D^{20}$ +26.6° (*c* 1.2, MeOH) (lit.,[10] $[\alpha]_D^{20}$ +30.0°, *c* 1.6, MeOH); $\nu_{max.}$(KBr) 3 340, 2 880, 2 620, and 2 480 cm^{-1}; δ_H 1.91 (2 H, m, 6-H_2), 2.64 (1 H, dt, *J* 10.8 and 6.5 Hz, 5-H), 3.24 (1 H, dt, 10.7 and 6.2 Hz, 5-H), 3.36 (1 H, m, 3-H), 3.80—4.15 (2 H, br s, 2 × OH),

* Picrolinic acid = 3-methyl-4-nitro-1-(*p*-nitrophenyl)pyrazol-5-ol.

3.85 (1 H, dd, *J* 15.5 and 1.9 Hz, 3-H), 3.99 (1 H, m, 8-H), 4.05 (1 H, dt, *J* 5.7 and 4.5 Hz, 7-H), 4.28 (2 H, s, 9-H_2), and 5.50 (1 H, d, *J* 1.5 Hz, 2-H); δ_C(50 MHz) 33.0 (C-6), 53.5 (C-5), 58.6 (C-9), 61.4 (C-3), 74.2 (C-7), 79.3 (C-8), 121.8 (C-2), and 140.9 (C-1); *m/z* 155 (M^+, 13%), 111, and 80 (100) (Found: M^+, 155.0951; C, 62.18; H, 8.51; N, 9.05%. $C_8H_{13}NO_2$ requires *M*, 155.0946; C, 61.91; H, 8.44; N, 9.03%).

The solution was acidified with 4M-hydrochloric acid solution and continuously extracted with diethyl ether for 48 h to yield (−)-viridifloric acid (440 mg, 80%), m.p. 120—121 °C [ethyl acetate–light petroleum (b.p. 40—60 °C) (1:1)] (lit.,[14] m.p. 119—120 °C); $[\alpha]_D^{20}$ −2.1° (*c* 3.0, water) (lit.,[14] $[\alpha]$ −2.2°, *c* 1.1, water); $\nu_{max.}$($CHCl_3$) 2 966, 2 566, and 1 705 cm^{-1}; δ_H(90 MHz) 0.93 (3 H, d, *J* 6.6 Hz, 6- or 7-H_3), 0.95 (3 H, d, *J* 6.6 Hz, 7- or 6-H_3), 1.21 (3 H, d, *J* 7.0 Hz, 4-H_3), 2.05 (1 H, m, 5-H), 4.02 (1 H, q, *J* 7.0 Hz, 3-H), 4.20 (2 H, br s, OH), and 8.70 (1 H, br s, CO_2H); δ_C(25 MHz) 16.0 and 16.8 (C-6 and -7), 17.5 (C-4), 32.0 (C-5), 71.2 (C-3), 83.8 (C-2), and 177.9 (C-1); *m/z* 118 (M^+, 44%), 103, 85, 57, 56, 45, and 43 (100) (Found: M^+, 118.0998; C, 51.73; H, 8.87%. $C_7H_{14}O_4$ requires *M*, 118.0995; C, 51.82; H, 8.70%).

General Procedure for the Synthesis of Dilactones [(**4**)—(**8**)].—The anhydride (0.35 mmol) was added to a solution of (+)-heliotridine (**1**) (0.30 mmol) in DME (10 ml) under N_2. After 24 h, triphenylphosphine (0.60 mmol) and 2,2′-dithiodipyridine (0.60 mmol) were added, and the mixture was stirred vigorously for 48 h. The homogeneous solution was transferred by syringe over 15 min to DME (150 ml) heated at reflux under N_2. Heating at reflux was continued for 14 h. The solution was cooled and concentrated to an oil which was dissolved in chloroform (10 ml). The chloroform solution was extracted with 1M-citric acid (4 × 8 ml). The acidic solution was washed with chloroform (4 × 10 ml) and made alkaline with conc. ammonia. The basic solution was extracted with chloroform (4 × 15 ml). The chloroform extracts were dried, filtered, and concentrated to an oil, which was purified by chromatography on basic alumina and elution with dichloromethane [(**4**) and (**6**)], or with increasing proportions (5—20%) of chloroform in dichloromethane [(**5**), (**7**), and (**8**)].

(+)-7,9-O,O′-(3,3-*Dimethylglutaryl*)*heliotridine* (**4**) was obtained as needles, m.p. 90—92 °C (cyclohexane) (55 mg, 61%); R_F 0.53; $[\alpha]_D^{20}$ +9.6° (*c* 2.7, $CHCl_3$); $\nu_{max.}$($CHCl_3$) 2 955, 2 923, 1 733, 1 415, and 1 258 cm^{-1}; δ_H 1.20 (6 H, s, 17-H_3 and 18-H_3), 1.89 (1 H, m, 6-H), 2.14 and 2.39 (2 H, AB system, *J* 13.3 Hz, 12- or 14-H_2), 2.18 and 2.29 (2 H, AB system, *J* 14.9 Hz, 14- or 12-H_2), 2.34 (1 H, m, 6-H), 2.59 (1 H, ddd, *J* 12.3, 9.7 and 5.5 Hz, 5-H), 3.37 (1 H, d, *J* 15.3 Hz, 3-H), 3.39 (1 H, dd, *J* 9.4 and 7.5 Hz, 5-H), 3.87 (1 H, dd, *J* 15.5 and 1.4 Hz, 3-H), 4.00 (1 H, m, 8-H), 4.44 and 5.05 (2 H, AB system, *J* 13.0 Hz, 9-H_2), 4.71 (1 H, ddd, *J* 12.7, 8.3, and 8.2 Hz, 7-H), and 5.62 (1 H, s, 2-H); δ_C(50 MHz) 29.5 and 30.4 (C-17 and -18), 32.9 (C-6), 33.6 (C-13), 44.1 and 44.9 (C-12 and -14), 54.1 (C-5), 59.8 and 62.4 (C-3 and -9), 74.2 (C-7), 79.7 (C-8), 125.9 (C-2), 136.5 (C-1), and 170.9 and 171.7 (C-11 and -15); *m/z* 279 (M^+, 18%), 137, 136, 120, 119 (100), 118, and 117 (Found: M^+, 279.1469; C, 64.65; H, 7.8; N, 4.5%. $C_{15}H_{21}NO_4$ requires *M*, 279.1470; C, 64.49; H, 7.58; N, 5.01%).

(+)-7,9-O,O′-(3,3-*Tetramethyleneglutaryl*)*heliotridine* (**5**) was obtained as prisms, m.p. 94—96 °C (cyclohexane) (56 mg, 57%); R_F 0.75; $[\alpha]_D^{20}$ +7.88° (*c* 3.3, $CHCl_3$); $\nu_{max.}$($CHCl_3$) 2 965, 2 915, 1 745, 1 438, and 1 178 cm^{-1}; δ_H 1.15—1.82 (8 H, m, 17-, 18-, 19-, and 20-H_2), 1.80—2.08 (1 H, m, 6-H), 2.30—2.45 (4 H, m, 12- and 14-H_2), 2.48—2.61 (1 H, m, 6-H), 2.85—3.00 (1 H, m, 5-H), 3.42 (1 H, d, *J* 15.0 Hz, 3-H), 3.57 (1 H, m, 5-H), 4.01 (1 H, d, *J* 14.8 Hz, 3-H), 4.08 (1 H, m, 8-H), 4.56 and 5.05 (2 H, AB system, *J* 13.5 Hz, 9-H_2), 4.80 (1 H, ddd, *J* 12.9, 8.1, and 7.5 Hz, 7-H), and 5.62 (1 H, s, 2-H); δ_C(50 MHz) 23.2 and 23.9 (C-18 and -19), 31.7 (C-6), 34.2 and 35.2 (C-17 and -20), 44.1 and 44.4 (C-12 and -14), 45.0 (C-13), 53.9 (C-5), 59.8 and 61.7 (C-3 and -9), 69.1 (C-7), 76.2 (C-8), 124.1 (C-2), 136.3 (C-1), and 171.2 and 171.9 (C-11 and -15); *m/z* 305 (M^+, 5%), 278, 277 (100), 199, 185, 136, 120, and 119 (Found: M^+, 305.1631; C, 66.65; H, 7.7; N, 4.85%. $C_{17}H_{23}NO_4$ requires *M*, 305.1627; C, 66.86; H, 7.59; N, 4.59%).

(+)-7,9-O,O′-(3,3-*Pentamethyleneglutaryl*)*heliotridine* (**6**) was obtained as needles, m.p. 101—102 °C (cyclohexane) (49.1 mg, 41%); R_F 0.69; $[\alpha]_D^{20}$ +6.0° (*c* 5.0, $CHCl_3$); $\nu_{max.}$($CHCl_3$) 2 934, 1 732, 1 454, 1 231, and 1 158 cm^{-1}; δ_H 1.33—1.72 (10 H, m, 17-, 18-, 19-, 20-, and 21-H_2), 1.80—2.05 (2 H, m, 6-H_2), 2.27 and 2.38 (2 H, AB system, *J* 15.1 Hz, 14- or 12-H_2), 2.30 and 2.43 (2 H, AB system, *J* 13.5 Hz, 12- or 14-H_2), 2.62 (1 H, ddd, *J* 12.5, 9.7, and 5.5 Hz, 5-H), 3.40 (1 H, d, *J* 14.5 Hz, 3-H), 3.41 (1 H, dd, *J* 9.7 and 7.5 Hz, 5-H), 3.85 (1 H, m, 3-H), 4.01 (1 H, m, 8-H), 4.41 and 5.15 (2 H, AB system, *J* 12.9 Hz, 9-H_2), 4.72 (1 H, ddd, *J* 10.8, 8.3, and 6.3 Hz, 7-H), and 5.66 (1 H, s, 2-H); δ_C(50 MHz) 21.5 and 21.6 (C-18 and -20), 25.8 (C-19), 32.2 (C-6), 36.4 (C-13), 37.0 and 37.9 (C-17 and C-21), 41.9 and 42.4 (C-12 and -14), 54.2 (C-5), 59.8 and 62.6 (C-3 and -9), 75.8 (C-7), 77.5 (C-8), 126.3 (C-2), 136.7 (C-1), 171.1 (C-15), and 177.1 (C-11); *m/z* 319 (M^+, 6%), 277, 149, 136, 120, and 119 (100) (Found: M^+, 319.1784; C, 67.5; H, 7.6; N, 4.55%. $C_{18}H_{25}NO_4$ requires *M*, 319.1783; C, 67.69; H, 7.89; N, 4.39%).

(+)-7,9-O,O′-(*Glutaryl*)*heliotridine* (**7**) was obtained as a pale yellow oil which could not be crystallised (20 mg, 41%); R_F 0.51; $[\alpha]_D^{20}$ +3.3° (*c* 1.2, $CHCl_3$); $\nu_{max.}$($CHCl_3$) 2 980, 2 935, 1 735, 1 278, and 1 183 cm^{-1}; δ_H 1.12—1.34 (2 H, m, 13-H_2), 1.43—1.60 (1 H, m, 6-H), 2.00—2.11 (2 H, m, 12- and 14-H), 2.24—2.64 (4 H, m, 5-, 6-, 12-, and 14-H), 3.41 (1 H, d, *J* 10.0 Hz, 3-H), 3.56 (1 H, m, 5-H), 4.05 (1 H, dd, *J* 10.1 and 1.5 Hz, 3-H), 4.18 (1 H, m, 8-H), 4.82 and 4.94 (2 H, AB system, *J* 15.0 Hz, 9-H_2), 5.01 (1 H, ddd, *J* 9.7, 7.7, and 7.5 Hz, 7-H), and 5.53 (1 H, s, 2-H); δ_C(50 MHz) 20.9 (C-13), 31.9 (C-6), 33.6 and 34.7 (C-12 and -14), 53.8 (C-5), 61.0 and 61.8 (C-3 and -9), 75.5 and 75.6 (C-7 and -8), 122.1 (C-2), 137.1 (C-1), and 172.6 and 172.7 (C-11 and -15); *m/z* 251 (M^+, 13%), 136, 120, 119, 93, and 59 (100) (Found: M^+, 251.1158; C, 61.9; H, 6.6; N, 5.85%. $C_{13}H_{17}NO_4$ requires *M*, 251.1157; C, 62.14; H, 6.82; N, 5.57%). The *picrolonate* had m.p. 192—194 °C (Found: C, 53.5; H, 4.6; N, 13.65. $C_{23}H_{25}N_5O_9$ requires C, 53.59; H, 4.85; N, 13.59%).

7,9-O,O′-[(2R,4S)-*Dimethylglutaryl*]*heliotridine* (**8a**) and 7,9-O,O′-[(2S,4R)-*Dimethylglutaryl*]*heliotridine* (**8b**) were obtained as a mixture of diastereoisomers which could not be separated (15.3 mg, 40%); R_F 0.55; $\nu_{max.}$($CHCl_3$) 2 963, 2 930, 1 734, 1 456, 1 260, and 1 184 cm^{-1}; δ_H 1.09—1.42 (6 H, m, 17- and 18-H_3), 1.46 (1/3 H, m, 13-H), 1.51 (2/3 H, m, 13-H), 1.95—2.63 (6 H, complex, 5-, 12-, 13-, 14-H, and 6-H_2), 3.30—3.45 (2 H, m, 3- and 5-H), 3.86 (2/3 H, m, 3-H), 3.92 (1/3 H, m, 3-H), 4.15 (2/3 H, m, 8-H), 4.30 (1/3 H, m, 8-H), 4.61 and 4.90 (2/3 H, AB system, *J* 14.0 Hz, 9-H_2), 4.73 and 4.90 (4/3 H, AB system, *J* 14.1 Hz, 9-H_2), 4.86 (1/3 H, m, 7-H), 5.08 (2/3 H, ddd, *J* 13.0, 8.9, and 6.3 Hz, 7-H), 5.45 (1/3 H, s, 2-H), and 5.55 (2/3 H, s, 2-H); δ_C(50 MHz) 17.9, 18.2, 19.4, and 19.6 (C-17 and -18), 31.9 and 32.0 (C-6), 38.8 and 39.7 (C-13), 39.2, 39.4, 39.6, and 39.7 (C-12 and -14), 53.8 and 53.9 (C-5), 60.5, 61.6, and 62.1 (C-3 and -9), 75.1, 75.2, 75.5, and 75.8 (C-7 and -8), 120.6 and 122.9 (C-2), 137.3 and 137.7 (C-1), and 174.8, 175.2, 175.6, and 175.7 (C-11 and -15); *m/z* 279 (M^+, 11%), 206, 136, 120, 119 (100), and 117 (Found: M^+, 279.1474; C, 64.65; H, 7.7; N, 4.85%. $C_{15}H_{21}NO_4$ requires *M*, 279.1470; C, 64.49; H, 7.58; N, 5.01%).

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