Pyrrolizidine Alkaloid Analogues. Synthesis of Eleven-membered Macrocyclic Diesters of Retronecine [1]

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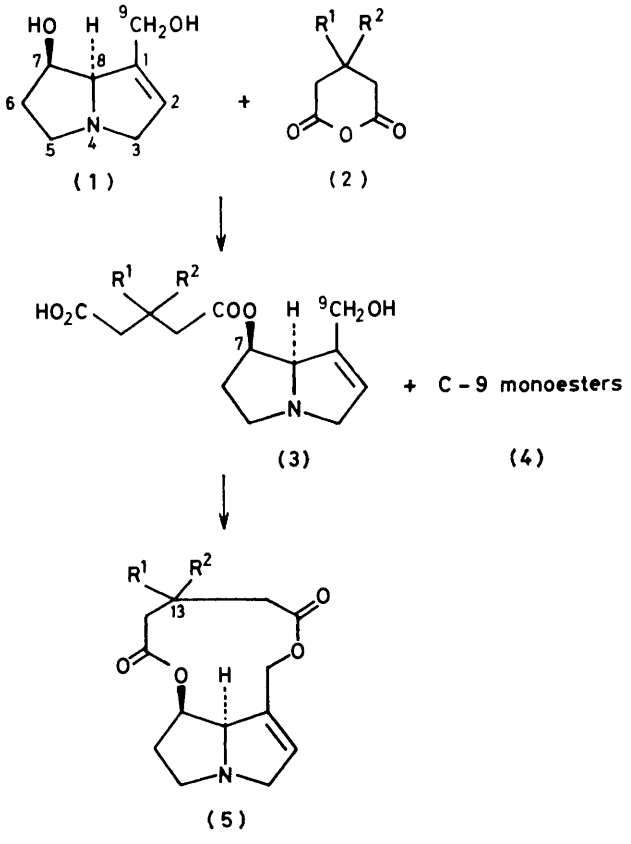
Treatment of (+)-retronecine (1) with 3,3-disubstituted glutaric anhydride derivatives (2) yielded mixtures of the retronecine 7- and 9-monoesters, which were assayed by ^{1}H n.m.r. spectrometry. Lactonisation of these mixtures was achieved by the Corey–Nicolaou method to give a range of eleven-membered macrocyclic pyrrolizidine diesters (5) with different substituents at C-13. The macrocyclic nature of these pyrrolizidine alkaloid analogues was established by comparison of their ^{1}H n.m.r. and mass spectra with those of natural pyrrolizidine alkaloids.

PYRROLIZIDINE alkaloids are important because of their widespread occurrence and the fact that many of them are hepatotoxic [2] and carcinogenic.[3] The structural features necessary for toxicity have been detailed by Mattocks.[4] The two most important features are the presence of a 1,2-double bond in the pyrrolizidine nucleus, and esterification at C-9. Increased toxicity is often observed when C-7 is also acylated. The most toxic pyrrolizidine alkaloids are those which combine all these features and contain a macrocyclic diester system, as in monocrotaline (6). More than twenty of these naturally occurring eleven-membered macrocyclic pyrrolizidine alkaloids have been isolated,[5] and most of them contain (+)-retronecine (1) as the base portion ('necine').



	R^1	R^2
a;	Me	Me
b;	[CH$_2$]$_4$	
c;	H	H
d;	Ph	Ph
e;	H	Me

SCHEME 1

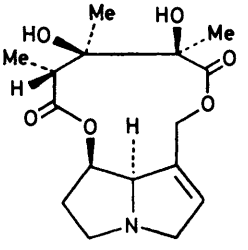
The total synthesis of macrocyclic pyrrolizidine diesters has been an outstanding challenge in this area. Several syntheses of (±)-retronecine have been reported,[6,7] and (+)-retronecine has been obtained by resolution.[6] A number of routes to acyclic ester derivatives of necines have been described.[8–10] In particular, Hoskins and Crout were able to achieve selective esterification at C-9 of retronecine (1) with simple acids using *N*,*N*′-dicyclohexylcarbodi-imide (DCCI), although significant amounts of the corresponding 7,9-diesters were also produced.[11] With bulky acids, this selective esterification was obtained by prior formation of the *N*-acylimidazoles using *N*,*N*′-carbonyldi-imidazole (CDI), before addition of the necine.

In order to achieve the total synthesis of eleven-membered macrocyclic pyrrolizidine diesters, we needed to produce 7- or 9-glutaryl monoesters of retronecine in high yield without any 7,9-diester formation. The formation of the macrocycle would then be attempted using one of the known methods for intramolecular lactonisation.[12] We chose to use symmetrically substituted glutaric anhydride derivatives (2a—d) to avoid the problem of diastereoisomer formation in the monoesters and cyclic diesters. Construction of the pyrrolizidine alkaloid analogues (5) would also be useful for investigation of structure–activity relationships.

RESULTS AND DISCUSSION

A supply of (+)-retronecine was obtained by hydrolysis of retrorsine, the major alkaloid in *Senecio isatideus* plants.[13] Preliminary experiments on the formation of monoesters were carried out with (+)-retronecine (1) and glutaric ester derivatives. The use of DCCI and CDI gave complex mixtures of products in low to moderate yield. These mixtures could not be separated, but it was evident from t.l.c. and mass spectral data that appreciable quantities of the acyclic 7,9-diesters of (+)-

retronecine were present, as observed earlier.[11] We therefore tried a different approach, utilising symmetrically substituted glutaric anhydrides. Treatment of equimolar amounts of a series of 3,3-disubstituted glutaric anhydride derivatives (2a—d) with (+)-retronecine (1) in chloroform gave a quantitative mixture of the corresponding 7- and 9-monoesters of (+)-retronecine [(3) and (4)] (Scheme 1). No 7,9-diester formation was observed, possibly because the initial zwitterionic monoester products usually precipitated. The proportions of 7- to 9-monoester were estimated from the ^{1}H n.m.r. spectra of each mixture. Characteristic signals for the C-7 methine and C-9 methylene protons



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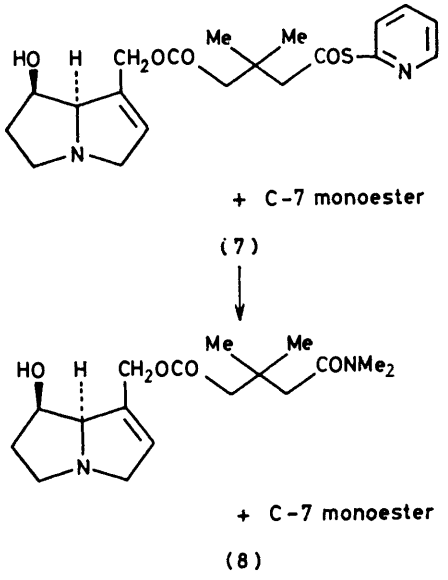
are observed when retronecine is acylated at C-7 or C-9.[14] The signals due to the C-2 vinyl proton also appear at different chemical shifts in the spectra of the two monoesters. Thus the ratio of 7- to 9-monoesters was estimated from the integrations of these signals. Except in the case of glutaric anhydride (2c), selective esterification at C-9 was observed. The relative positions of the signals for the C-2, C-7, and C-9 protons in the ^{1}H n.m.r. spectra of the monoester mixture (3d) and (4d) derived from (+)-retronecine and 3,3-diphenylglutaric anhydride were different from the other examples studied. The assignment of these signals was based on their line shapes, and was confirmed by decoupling experiments.

The widely used Corey–Nicolaou double activation method was selected for use in the lactonisation step.[12,15] The main problem was that of the insolubility of the zwitterionic monoester mixtures in the high-boiling inert solvents generally employed in this procedure, and in more polar solvents such as methylene chloride, acetone, or acetonitrile. It was eventually found that the 7- and 9-monoesters of 3,3-dimethylglutaric acid [(3a) and (4a)] were soluble in dimethylformamide. Cyclisation was achieved by slow addition of the pyridine-2-thiol esters of this monoester mixture to refluxing dimethylformamide, followed by 20 h at reflux. The crude product was subjected to acid/base recycling, and one major product, R_F 0.58, was then observed on t.l.c. Preparative t.l.c. gave a 50% yield of 13,13-dimethyl-1,2-didehydrocrotalanine[16] (5a), as an oil, which was characterised as its picrate, picrolonate, and hydrobromide derivatives. The i.r. spectrum of the free base (5a) in carbon tetrachloride contained a single ester carbonyl absorption at 1 738 cm^{-1}, whereas a value of 1 726 cm^{-1} was observed for the monoester mixture. An absorption at 1 737 cm^{-1} has been recorded for the natural eleven-membered macrocyclic alkaloid monocrotaline (6).[17] A typical fragmentation pattern for a macrocyclic pyrrolizidine diester was observed in the mass spectrum of the free base (5a), including an ion at *m/z* 136 characteristic of pyrrolizidine diesters.[18] The key feature in the ^{1}H n.m.r. spectrum of (5a) is an AB quartet (J 12 Hz) due to the non-equivalent protons at C-9. The chemical shift difference between these protons [$\Delta\delta$(H-9)] is 1.24 p.p.m. This combination of spectral data is considered convincing evidence for the formation of an eleven-membered macrocyclic diester of (+)-retronecine.

A more polar minor component was also separated from the cyclisation products by preparative t.l.c. This is formulated as a mixture of the *N,N*-dimethylamides (8) of the 7- and 9-monoesters of retronecine with 3,3-dimethylglutaric acid. The mixture could not be separated by t.l.c. Its i.r. spectrum showed an amide band at 1 690 cm^{-1}, and *N*-Me signals were observed at δ 2.90 and 3.00 in the ^{1}H n.m.r. spectrum. The *N,N*-dimethylamides (8) are presumably formed by reaction of dimethylamine (from breakdown of dimethylformamide) with the pyridine-2-thiol esters (7) (Scheme 2). The yield of this by-product varied (10—30%).



SCHEME 2

The formation of the undesirable products (8) suggested that an alternative solvent should be sought for the cyclisation step. The use of 1,2-dimethoxyethane resulted in a yield of 64% for (5a) with no side products evident. A further improvement in yields (consistently 80—85% in several runs) was achieved by not concentrating the suspension of monoesters [(3a) and (4a)] formed in chloroform solution. The pyridine-2-thiol esters were prepared by adding the reagents to this

suspension and stirring to effect dissolution. The cyclisation was then carried out in refluxing chloroform.

Three more macrocyclic pyrrolizidine diesters (5b—d) were prepared as oils (60—75% yields) and characterised as their picrates, except for the unstable diphenyl analogue (5d) for which no solid derivative could be prepared. All the free bases gave mass spectra typical of the macrocyclic diester alkaloids. The Δδ(H-9) values for these macrocyclic bases were 1.23 p.p.m. for the tetramethylene derivative (2b), 0.62 p.p.m. for the parent compound (2c), and 0.0 p.p.m. for the diphenyl derivative (2d).

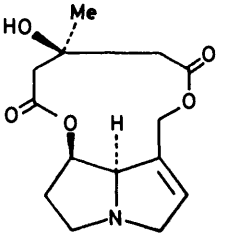
Reaction of 3-methylglutaric anhydride with (+)-retronecine yielded the two diastereoisomers of the macrocycle (5e) as expected. Although these compounds could not be separated by t.l.c., the 360 MHz ^{1}H n.m.r. spectrum permitted key signals of the two epimers to be distinguished. They were present in the ratio 2 : 1. The Δδ(H-9) values were 0.51 and 0.99 p.p.m.

The Δδ(H-9) values of macrocyclic pyrrolizidine diesters have been used to assign ring size. Thus, for eleven-membered alkaloids, values of 0.0—0.92 p.p.m. have been recorded; and 1.25—1.53 p.p.m. for twelve-membered alkaloids.[5] These values are believed to reflect the different conformations of the diacid portion in the two different ring sizes. From *X*-ray data of the alkaloids investigated, the ester carbonyl groups of eleven-membered alkaloids are synperiplanar and directed below the plane of the ring, while for twelve-membered compounds the ester carbonyl groups are antiperiplanar.[5] In view of the unusually high values observed for Δδ(H-9) in two of our macrocyclic pyrrolizidine diesters [1.24 for (5a) and 1.23 p.p.m. for (5b)], caution should be exercised in assigning ring size to pyrrolizidine alkaloids purely from the values for Δδ(H-9). The high values for (5a) and (5b) may be due to unusual conformations adopted by these particular macrocyclic diesters.

An unusual conformation is also apparent for the diphenyl analogue (5d) from its ^{1}H n.m.r. spectrum. The protons at C-9 are magnetically equivalent, but like the C-2 protons they are shielded; chemical shifts differ from the normal values by 0.35 and 0.5 p.p.m., respectively. In addition, two of the aromatic protons are deshielded (chemical shift differences 0.41 and 1.28 p.p.m.). These two protons show mutual *meta*-coupling, and further coupling with the remaining aromatic protons (δ 7.23 p.p.m.). These data indicate that one of the aromatic rings is close to the 1,2-double bond of the retronecine moiety.

The hydrobromide salt of the dimethyl analogue (5a) has been tested for toxicity on rats by Mattocks.[19] It is readily metabolised to its toxic pyrrole derivative, and has a hepatotoxicity similar to that of monocrotaline (6), a common macrocyclic pyrrolizidine alkaloid. The analogue (5a) will therefore be useful for further toxicity studies, particularly as its synthesis in radioactive form is now feasible.

The foregoing general method for formation of macrocyclic pyrrolizidine diesters has been successfully applied by us to the synthesis of dicrotaline (9).[20] Huang and Meinwald recently reported a different approach to the synthesis of crobarbatine acetate and a diastereoisomer (10), but they were unable to decide



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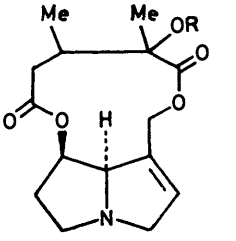

(10) R = Ac
(11) R = H

which of their two products was a derivative of natural crobarbatine (11).[21] Total synthesis of other macrocyclic pyrrolizidine esters will now be attempted.

EXPERIMENTAL

M.p.s were measured with a Kofler hot-stage apparatus. Organic solutions were dried with anhydrous $MgSO_4$, and solvents were evaporated off under reduced pressure below 40 °C. N.m.r. spectra were run for solutions in deuteriochloroform unless otherwise stated, with tetramethylsilane as internal standard. Mass spectra were obtained with an A.E.I. MS12 spectrometer. Optical rotations were measured with a Perkin-Elmer 141 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), and the bases were located by oxidation with *o*-chloranil, followed by treatment with Ehrlich's reagent.[22]

(+)-*Retronecine* (1).—A supply of (+)-retronecine was obtained by hydrolysis of retrorsine, extracted from *Senecio isatideus* plants as previously described.[13]

(+)-*13,13-Dimethyl-1,2-didehydrocrotalanine* (5a).—*Method* 1. A solution of 3,3-dimethylglutaric anhydride (2a) (46 mg, 0.32 mmol) in chloroform (5 ml) was added to a solution of (+)-retronecine (1) (50 mg, 0.32 mmol) in chloroform (5 ml), and the mixture was stirred at room temperature for 12 h. The solvent was removed to give a mixture of 7- (3a) and 9-*O*-(hydrogen 3,3-dimethylglutaryl)-retronecine (4a) as a gum (96 mg, 100%), $\nu_{max.}$ ($CHCl_3$) 3 300, 3 000, and 1 726 cm^{-1}; δ(CD_3OD) 4.52 (m, H-7 of C-7 ester), 4.71 (s, H-9 of C-9 ester), 5.68 (m, H-2 of C-7 ester), 5.76 (m, H-2 of C-9 ester); the integrations for these signals showed that the ratio of (3a) to (4a) varied from 1 : 2 to 1 : 7 in different runs. Triphenylphosphine (105 mg, 0.4 mmol) and 2,2′-dithiobipyridyl (88 mg, 0.4 mmol) were added to a solution of the 7- and 9-monoesters of retronecine [(3a) and (4a)] (96 mg, 0.32 mmol) in dimethylformamide (15 ml) under argon, and the mixture was stirred at room temperature for 12 h. The resulting yellow solution was diluted with dimethylformamide (10 ml) and added over 6 h by syringe to dimethylformamide (15 ml) heated at reflux under argon. When the addition was complete, the mixture was heated at reflux for a further

20 h. The cooled solution was concentrated to an oil, which was dissolved in M-sulphuric acid (10 ml). The acidic solution was washed with chloroform (2 × 10 ml), and then basified with conc. ammonia (10 ml). The basic solution was extracted with chloroform (4 × 10 ml). The combined chloroform extracts were washed with M-sodium hydroxide (5 ml) and water (2 × 10 ml), dried, filtered, and concentrated to yield an oil which contained two components, R_F 0.58 and 0.3 to 0.4. Separation of the faster running component by preparative t.l.c. or by vacuum liquid column chromatography [23] on silica gel at 18 mmHg with 5% v/v methanol–chloroform as eluant gave 13,13-*dimethyl*-1,2-*didehydrocrotalanine* (5a) as an oil (45 mg, 50%), $[\alpha]_D^{22}$ +42.5° (*c* 4.40 in $CHCl_3$); $\nu_{max.}$ (CCl_4) 1 735 and 1 655 cm^{-1}; δ 1.18 (3 H, s, Me), 1.22 (3 H, s, Me), 2.03 and 2.22 (4 H, ABq, *J* 13.5 Hz, H-12 and H-14), 2.10—2.40 (2 H, complex, H-6), 2.50—3.10 (2 H, complex, H-5), 3.30—3.89 (2 H, complex, H-3), 4.35 (1 H, m, H-8), 5.14 (1 H, m, H-7), 4.08 and 5.32 (2 H, ABq, *J* 12 Hz, H-9), 5.88 (1 H, m, H-2); *m/z* 279 (M^+), 137, 136, 120, 119, 94, 93, and 80 (Found: M^+, 279.1469. $C_{15}H_{21}NO_4$ requires *M*, 279.1470). The *picrate* had m.p. 191—192 °C (EtOH) (Found: C, 49.45; H, 4.85; N, 11.3. $C_{21}H_{24}N_4O_{11}$ requires C, 49.6; H, 4.7; N, 11.0%). The *picrolonate* had m.p. 232—234 °C (decomp.) ($CHCl_3$) (Found: C, 55.4; H, 5.45; N, 12.8. $C_{25}H_{29}N_5O_9$ requires C, 55.25; H, 5.35; N, 12.9%). The *hydrobromide* had m.p. 208—210 °C (EtOH) (Found: C, 50.1; H, 5.85; N, 3.8. $C_{15}H_{22}BrNO_4$ requires C, 50.0; H, 6.1; N, 3.9%).

The more polar component of the mixture was separated by preparative t.l.c. as an oily mixture of the *N,N*-dimethylamides (8) of 7- and 9-*O*-(hydrogen 3,3-dimethylglutaryl)-retronecine (10—30% yield in different runs), $\nu_{max.}$ ($CHCl_3$) 3 300, 1 725, and 1 690 cm^{-1}; δ 1.00 (6 H, br s, Me_2C), 2.90 (3 H, s, MeN), 3.00 (3 H, s, MeN), 5.70 (m, H-2 of C-7 ester), and 5.81 (m, H-2 of C-9 ester) (plus usual complex pattern for retronecine); *m/z* 324 (M^+), 137, 136, 120, 119, 113, 99, 94, and 93 (Found: M^+, 324.2044. $C_{17}H_{28}N_2O_4$ requires *M*, 324.2050).

Method 2. A mixture of the monoesters (3a) and (4a), prepared as in Method 1, on a 0.32 mmol scale, was dissolved in 1,2-dimethoxyethane (10 ml). The pyridine-2-thiol esters were also formed as before, and then added to 1,2-dimethoxyethane (50 ml) at reflux under argon over 4 h. The mixture was heated at reflux for a further 10 h and then cooled. Work-up as in Method 1 gave 13,13-dimethyl-1,2-didehydrocrotalanine (5a) as an oil (54 mg, 64%).

Method 3. A mixture of the monoesters (3a) and (4a) was formed as in Method 1 on a 0.32 mmol scale. The suspension of monoesters in chloroform was not concentrated, but used directly for the preparation of the pyridine-2-thiol esters. Vigorous stirring was required to effect dissolution. The chloroform solution was then added to refluxing chloroform (50 ml) under argon over 4 h, and then the mixture was heated at reflux for 12 h. Work-up as in Method 1 gave 13,13-dimethyl-1,2-didehydrocrotalanine as an oil (73 mg, 84%).

(+)-13,13-*Tetramethylene*-1,2-*didehydrocrotalanine* (5b).—Solutions of (+)-retronecine (50 mg, 0.32 mmol) in chloroform (2 ml) and 3,3-tetramethyleneglutaric anhydride (2b) (55 mg, 0.32 mmol) in chloroform (2 ml) were mixed and stirred at room temperature for 18 h. The solvent was removed from a sample of this solution to yield a mixture of 7- (3b) and 9-*O*-(hydrogen 3,3-tetramethyleneglutaryl)-retronecine (4b) as an oil, $\nu_{max.}$ ($CHCl_3$) 3 300, 3 010, and 1 725 cm^{-1}; δ(CD_3OD) 4.50 (m, H-7 of C-7 ester), 4.68 (s, H-9 of C-9 ester), 5.60 (m, H-2 of C-7 ester), and 5.72 (m, H-2 of C-9 ester); from the integrations for these signals, the ratio of (3b) to (4b) was 1 : 3. The pyridine-2-thiol esters of (3b) and (4b) were formed in chloroform, and the mixture was cyclised in chloroform as in Method 3. The products were purified as in Method 1 to give 13,13-*tetramethylene*-1,2-*didehydrocrotalanine* (5b) as an oil, R_F 0.62 (60 mg, 60%), $[\alpha]_D^{22}$ +45.1° (*c* 1.0 in $CHCl_3$); $\nu_{max.}$ (CCl_4) 1 730, and 1 675 cm^{-1}; δ 1.2—2.7 (16 H, complex), 3.2—3.9 (2 H, complex, H-3), 4.32 (1 H, m, H-8), 5.13 (1 H, m, H-7), 4.10 and 5.33 (2 H, ABq, *J* 13 Hz, C-9), and 5.89 (1 H, m, H-2); *m/z* 305 (M^+), 137, 136, 120, 119, 94, 93, and 80 (Found: M^+, 305.1622. $C_{17}H_{23}NO_4$ requires *M*, 305.1626). The *picrate* had m.p. 205—208 °C (decomp.) (EtOH) (Found: C, 51.4; H, 4.95; N, 9.95. $C_{23}H_{26}N_4O_{11}$ requires C, 51.7; H, 4.85; N, 10.3%).

(+)-1,2-*Didehydrocrotalanine* (5c).—Solutions of (+)-retronecine (15.5 mg, 0.1 mmol) in chloroform (5 ml) and glutaric anhydride (2c) (11.4 mg, 0.1 mmol) in chloroform (5 ml) were mixed, and stirred vigorously for 6 h at room temperature. A sample of the finely divided suspension formed was concentrated to yield a mixture of 7- (3c) and 9-*O*-(hydrogen glutaryl)retronecine (4c) as an oil, $\nu_{max.}$ ($CHCl_3$) 3 310, 3 000, and 1 720 cm^{-1}; δ[$(CD_3)_2SO$] 4.50 (m, H-7 of C-7 ester), 4.62 (s, H-9 of C-9 ester), 5.70 (m, H-2 of C-7 ester), and 5.80 (m, H-2 of C-9 ester); from the integrations for these signals the ratio of (3c) to (4c) was 1 : 1. The pyridine-2-thiol esters of (3c) and (4c) were formed in chloroform (2.5 mol equiv. of reagents were used) with vigorous stirring for 12 h. The clear solution obtained was added to refluxing chloroform as in Method 3. The cyclised products were purified as in Method 1 to give 1,2-*didehydrocrotalanine* (5c) as an oil, R_F 0.52 (20 mg, 74%), $[\alpha]_D^{20}$ +39.0° (*c* 1.0 in $CHCl_3$); $\nu_{max.}$ (CCl_4) 1 732 and 1 605 cm^{-1}; δ 1.90—2.20 (4 H, complex, H-6 and H-13), 2.22—2.49 (4 H, complex, H-12 and H-14), 2.50—2.83 and 3.20—3.45 (2 H, complex, H-5), 3.45—4.01 (2 H, complex, H-3), 4.41 (1 H, m, H-8), 4.34 and 4.96 (2 H, ABq, *J* 12 Hz, H-9), 5.32 (1 H, m, H-7), 5.97 (1 H, m, H-2); *m/z* 251 (M^+), 137, 136, 120, 119, 94, 93, and 80 (Found: M^+, 251.1156. $C_{13}H_{17}NO_4$ requires *M*, 251.1157). The *picrate* had m.p. 210—212 °C (decomp.) (EtOH) (Found: C, 47.3; H, 4.05; N, 11.4. $C_{19}H_{20}N_4O_{11}$ requires C, 47.5; H, 4.15; N, 11.65%).

(+)-13,13-*Diphenyl*-1,2-*didehydrocrotalanine* (5d).—3,3-Diphenylglutaric anhydride (2d) was prepared by the method of Bruice and Bradbury; [24] m.p. 149—150 °C (lit.,[24] 147—148 °C). Solutions of (+)-retronecine (15.5 mg, 0.1 mmol) in chloroform (2 ml) and 3,3-diphenylglutaric anhydride (26.6 mg, 0.1 mmol) in chloroform (2 ml) were mixed and stirred at room temperature for 6 h. A sample of the solution was concentrated to give a mixture of 7- (3d) and 9-*O*-(hydrogen 3,3-diphenylglutaryl)retronecine (4d) as an oil, $\nu_{max.}$ (Nujol) 3 220, 1 735, 1 500, and 910 cm^{-1}; δ(CD_3OD) 4.74 (m, H-9 of C-9 ester), 5.00 (m, H-7 of C-7 ester), 5.55 (m, H-2 of C-9 ester), and 5.60 (m, H-2 of C-7 ester); from integration the ratio of (3d) to (4d) was 1 : 7. The pyridine-2-thiol esters of (3d) and (4d) were formed and cyclised in chloroform as in Method 3. The products were purified as in Method 1 to give 13,13-*diphenyl*-1,2-*didehydrocrotalanine* (5d) as an oil, R_F 0.60 (32 mg, 75%), $[\alpha]_D$ +17° (*c* 1.0 in $CHCl_3$); $\nu_{max.}$ (CCl_4) 1 740, 1 578, 1 450, and 1 424 cm^{-1}; δ 1.95 (2 H, complex, H-6), 2.50—2.95 (1 H, complex, H-3), 3.38—3.58 (4 H, complex,

2 CH_2), 3.95—4.13 (4 H, complex, H-3, H-5, and H-8), 4.45 (2 H, s, H-9), 5.05 (1 H, complex, H-7), 5.40 (1 H, s, H-2), 7.32 (8 H, complex, ArH), 7.62 (1 H, complex, ArH), and 8.51 (1 H, complex, ArH); *m/z* 403 (M^+), 137, 136, 120, 119, 94, and 93 (Found: M^+, 403.1764. $C_{25}H_{25}NO_4$ requires *M*, 403.1784).

(13R)- *and* (13S)-13-*Methyl*-1,2-*didehydrocrotalanine* (5e). —Solutions of (+)-retronecine (78 mg, 0.5 mmol) in chloroform (5 ml) and 3-methylglutaric anhydride (2e) (64 mg, 0.5 mmol) in chloroform (5 ml) were mixed and stirred at room temperature for 4 h. The precipitate was dried to give 9-*O*-[hydrogen (3*RS*)-3-methylglutaryl]-retronecine (4e) as an oil, (142 mg, 100%), $\nu_{max.}$ (Nujol) 3 300, 3 000, and 1 730 cm^{-1}; $\delta(CD_3OD)$ 1.16 (3 H, d, *J* 8 Hz, Me), 4.70 (2 H, s, H-9), and 6.79 (1 H, br, s, H-2); no signals corresponding to esterification at C-7 were observed. The epimers (4e) were dissolved in dimethylformamide (1 ml), and triphenylphosphine (131 mg, 0.5 mmol) and 2,2′-dithiobipyridyl (110 mg, 0.5 mmol) were added. The mixture was stirred at room temperature for 16 h and added dropwise over 6 h to refluxing 1,2-dimethoxyethane (100 ml) under argon. The solution was then heated at reflux for a further 8 h. Work-up and separation as in Method 1 yielded a mixture of (13*R*)- and (13*S*)-13-methyl-1,2-didehydrocrotalanine (5e) as an oil, R_F 0.55 (40 mg, 30%), $\nu_{max.}$ ($CHCl_3$) 1 732 and 1 634 cm^{-1}; δ(360 MHz) (major isomer) 1.11 (3 H, d, *J* 7 Hz, Me), 4.53 (1 H, m, H-8), 4.30 and 4.81 (2 H, ABq, *J* 12 Hz, H-9), 5.39 (1 H, m, H-7), and 5.95 (1 H, d, *J* 0.1 Hz, H-2); (minor isomer) 1.23 (3 H, br, s, Me), 4.49 (1 H, m, H-8), 4.03 and 5.20 (2 H, ABq, *J* 12 Hz, H-9), 5.15 (1 H, m, H-7), and 5.92 (1 H, br, s, H-2); the ratio of major to minor isomers was 2 : 1 (from integration): *m/z* 265 (M^+), 137, 136, 121, 122, and 93 (Found: M^+, 265.1322. $C_{14}H_{19}NO_4$ requires *M*, 265.1312).

A second more polar component was separated by preparative t.l.c. (R_F 0.25) as an oily mixture of the *N*,*N*-dimethylamides of 9-*O*-[hydrogen (3*R*)- and (3*S*)-3-methylglutaryl]retronecine (40 mg, 25%); $\nu_{max.}$ ($CHCl_3$) 3 300, 1 725, and 1 685 cm^{-1}; δ 1.00 (3 H, d, *J* 7 Hz, MeC), 2.90 (3 H, s, MeN), 2.98 (3 H, s, MeN), 4.70 (2 H, br, s, H-9), and 5.82 (1 H, br, s, H-2) (plus the usual complex signals for retronecine); *m/z* 310 (M^+), 137, 136, 120, 119, 94, 93, and 80 (Found: M^+, 310.1890. $C_{16}H_{26}N_2O_4$ requires *M*, 310.1892).

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