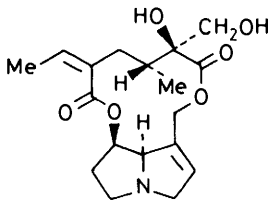
Pyrrolizidine Alkaloid Analogues. Synthesis of Ten-membered Macrocyclic Diesters of (+)-Retronecine

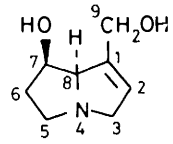
Michael Burton and David J. Robins*
Department of Chemistry, University of Glasgow, Glasgow G12 8QQ

The first synthesis of ten-membered macrocyclic pyrrolizidine diesters [(**3**)—(**6**)] has been achieved by treatment of (+)-retronecine (**2**) with succinic anhydride, (±)-*trans*-cyclohexane-1,2-dicarboxylic anhydride, and phthalic anhydride respectively, followed by lactonisation *via* the pyridine-2-thiol esters.

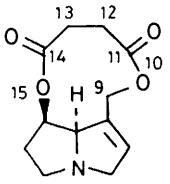
The importance of pyrrolizidine alkaloids is due to their widespread occurrence and their known hepatotoxicity and, in some cases, carcinogenicity.[1,2] The structural features regarded as necessary for toxicity are the presence of a 1,2-double bond in the pyrrolizidine nucleus [as in compound (**1**)] and esterification at C-9.[3] The most toxic pyrrolizidine alkaloids contain a dilactone, as in retrorsine (**1**). Steric hindrance around the ester groups in compound (**1**) enhances the toxicity by reducing the susceptibility of the alkaloid to detoxification by hydrolysis.[4] Retronecine (**2**) is the most common base portion and has been found naturally as part of 11-, 12-, 13-, and 14-membered rings.[2] The synthesis of macrocyclic pyrrolizidine alkaloids has been limited to (+)-dicrotaline,[5] (±)-integerrimine,[6] fulvine and crispatine,[7] and the *O*-acetyl derivative of crobarbatine.[8] A series of 11-membered analogues has also been prepared.[9] No pyrrolizidine alkaloids containing ten-membered rings have so far been discovered, although succinic acid derivatives are common plant constituents. In order to develop our understanding of the relationship between structure and toxicity of pyrrolizidine alkaloids and analogues, we sought to prepare ten-membered macrocyclic diesters of (+)-retronecine.



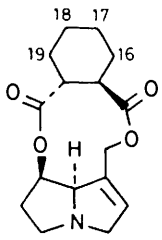
(**1**)



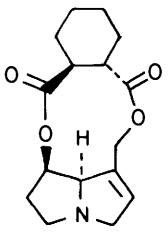
(**2**)



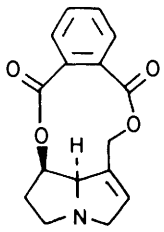
(**3**)



(**4**)



(**5**)



(**6**)

Results and Discussion

(+)-Retronecine (**2**) was obtained by alkaline hydrolysis[10] of retrorsine (**1**), which is the major alkaloid produced by *Senecio isatideus* plants.[11] Treatment of (+)-retronecine with succinic anhydride in dry 1,2-dimethoxyethane gave a mixture of the 9- and 7-succinyl monoesters of (+)-retronecine. Lactonisation of this mixture was achieved by the Corey–Nicolaou method,[12] by heating the corresponding pyridine-2-thiol esters at reflux in 1,2-dimethoxyethane. Purification of the reaction products by column chromatography on basic alumina gave a 79% yield of the ten-membered pyrrolizidine alkaloid analogue (**3**) as needles, m.p. 122—123 °C, $[\alpha]_D^{20}$ +11.8° ($CHCl_3$). An accurate mass measurement on the base (**3**) gave a molecular formula of $C_{12}H_{15}NO_4$. In addition, the mass spectral fragmentation pattern was typical of a macrocyclic retronecine diester[1] with peaks at *m/z* 237 (M^+), 138, 137, 136, 120, 119, 94, 93, and 80. In the ^1H n.m.r. spectrum of compound (**3**) in deuteriochloroform, an AB system is visible at δ 4.03 and 5.37 (*J* 12 Hz) due to the methylene protons at C-9. The chemical shift difference (Δδ) of 1.34 p.p.m. between these protons in compound (**3**) is similar to values observed for 12-membered diesters of (+)-retronecine,[1] but is much higher than the values recorded for 11-membered alkaloids (Δδ 0—0.92 p.p.m.), with the exception of dicrotaline (Δδ 1.24 p.p.m.).[5]

In order to test the scope of this cyclisation procedure, and to obtain an analogue with more steric crowding around the ester groups, we investigated the lactonisation of a more substituted diacid. The reaction of (±)-*trans*-cyclohexane-1,2-dicarboxylic anhydride with (+)-retronecine gave a monoester mixture, which was lactonised by heating the pyridine-2-thiol esters at reflux in 1,2-dimethoxyethane. Purification by column chromatography gave a mixture of the diastereoisomeric cyclised products (**4**) and (**5**) in 14% yield as an oil. These isomers could not be separated by t.l.c. on silica gel and alumina in a variety of solvent systems. However, the ^1H and ^{13}C n.m.r. spectra clearly demonstrated that the two diastereoisomers are present in a ratio of *ca.* 3.8:1. An accurate mass measurement of the mixture of compounds (**4**) and (**5**) gave a molecular formula of $C_{16}H_{21}NO_4$, and the fragmentation pattern was similar to the succinyl diester (**3**). The major component of the mixture of diastereoisomers was identified as compound (**4**) by hydrolysis

of the mixture with barium hydroxide to give *trans*-cyclohexane-1,2-dicarboxylic acid in 99% yield, $[\alpha]_D^{21}$ − 12.3° (acetone) {lit.,[13] for the (1*S*,2*S*)-diacid $[\alpha]_D^{30}$ + 22.3°}. This indicates an optical purity of *ca.* 55% for the (1*R*,2*R*)-*trans*-cyclohexane-1,2-dicarboxylic acid, which corresponds to a ratio of 3.5:1 for the enantiomeric acids. The major diastereoisomer formed in the lactonisation is therefore (**4**). Again a large difference was observed for the chemical shifts of the protons at C-9 of the mixture in deuteriochloroform. The major isomer (**4**) has a Δδ value of 1.32 p.p.m., while the minor isomer (**5**) exhibits a Δδ of 1.28 p.p.m.

Finally, a ten-membered pyrrolizidine alkaloid analogue containing an aromatic diacid was prepared. Lactonisation of the monoester mixture formed from phthalic anhydride and (+)-retronecine was carried out by heating the corresponding pyridine-2-thiol esters at 140 °C in dimethylformamide. No cyclisation was observed in other solvents tested including 1,2-dimethoxyethane. A 16% yield of the ten-membered macrocyclic diester (**6**) was obtained as needles, m.p. 145—146 °C, $[\alpha]_D^{19}$ + 239° ($CHCl_3$). An accurate mass measurement gave a molecular formula of $C_{16}H_{15}NO_4$, and the mass spectral fragmentation pattern was similar to that of compounds (**3**)—(**5**). Despite the difference in the geometry of the macrocyclic system caused by the aromatic ring, a Δδ value of 1.28 p.p.m., similar to those observed for compounds (**3**)—(**5**), was recorded for the C-9 protons in the ^{1}H n.m.r. spectrum of the phthalate diester (**6**) in deuteriochloroform (ABq, δ 4.27 and 5.55, *J* 12 Hz).

Lactonisation of (+)-retronecine with a range of structural types of 1,2-diacids has been achieved. The toxicity of these new ten-membered pyrrolizidine alkaloid analogues can now be established, particularly as their synthesis in radioactive form is feasible.

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 50 °C. N.m.r. spectra were recorded with a Bruker WP-200 SY spectrometer operating at 200 MHz or at 360 MHz with a Bruker WH-360 spectrometer. Spectra were recorded for solutions in deuteriochloroform unless otherwise stated, with tetramethylsilane as internal standard. Mass spectra were obtained with A.E.I. MS 12 or 902 spectrometers. Optical rotations were measured with an Optical Activity Ltd. AA-100 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.[14] 1,2-Dimethoxyethane (DME) was dried by distillation from potassium hydroxide and then from sodium–benzophenone under argon immediately prior to use. Dimethylformamide (DMF) was dried utilising 3A molecular sieves as detailed by Burfield and Smithers.[15]

(+)-*Retronecine* (**2**).—Extraction of *Senecio isatideus* plants as described previously yielded retrorsine (**1**), from which (+)-retronecine (**2**) was obtained by alkaline hydrolysis.[10,11]

(+)-7,9-O,O-(*Succinyl*)*retronecine* (**3**).—Succinic anhydride (37.1 mg, 0.371 mmol) was added to a stirred solution of (+)-retronecine (54.9 mg, 0.354 mmol) in DME (10 ml) under argon. After *ca.* 5 min a colourless precipitate formed. After 5 h, triphenylphosphine (185.4 mg, 0.707 mmol) and 2,2′-dithiodipyridine (155.6 mg, 0.707 mmol) were added and stirring was continued for 2 days. The clear yellow solution was then added during 15 min by syringe to DME (60 ml) heated at reflux under argon. After the addition was complete, the heating at reflux was continued for a further 5 h. The cooled solution was concentrated to an oil under reduced pressure and dissolved in chloroform (5 ml). The chloroform solution was cooled (ice) and extracted with cold 1M-citric acid (4 × 3 ml). The acidic solution was washed with chilled chloroform (6 × 5 ml) then basified (>pH 10) with conc. ammonia and extracted with chloroform (4 × 6 ml). The basic chloroform extracts were dried, filtered, and concentrated to give crude cyclised product (**3**) as an oil which was purified by application to a basic alumina column and elution with 20% v/v chloroform in dichloromethane. 7,9-O,O-(*Succinyl*)*retronecine* (**3**) was obtained as long colourless needles (66.1 mg, 79%), R_F 0.56; m.p. 122—123 °C (hexane); $[\alpha]_D^{20}$ +11.8° (*c* 0.509 in $CHCl_3$); $\nu_{max.}$($CHCl_3$) 1 738 cm^{-1}; δ_H (360 MHz) 1.98—2.13 (2 H, m, 6-H_2), 2.44—2.56 (4 H, m, 12- and 13-H_2), 2.59 (1 H, m, 5β-H), 3.29 (1 H, m, 5α-H), 3.44 (1 H, m, 3β-H), 3.87 (1 H, m, 3α-H), 4.03 (1 H, ABq, *J* 12 Hz, 9-H), 4.31 (1 H, m, 8-H), 5.37 (1 H, ABq, *J* 12 Hz, 9-H), 5.37 (1 H, m, 7-H), and 5.95 (1 H, br s, 2-H); δ_C (50 MHz; C_6D_6) 32.8 and 33.1 (C-12 and -13), 34.7 (C-6), 54.2 (C-5), 60.0 (C-9), 61.8 (C-3), 74.9 and 77.4 (C-7 and -8), 132.6 (C-1), 133.8 (C-2), and 171.1 (C-11 and -14); *m/z* 237 (M^+) (21%), 138, 137, 136, 120, 119, 94, 93 (100%), and 80 (Found: M^+, 237.1004. $C_{12}H_{15}NO_4$ requires *M*, 237.1001) (Found: C, 60.55; H, 6.3; N, 5.9. $C_{12}H_{15}NO_4$ requires C, 60.75; H, 6.4; N, 5.9%).

7,9-O,O-[(1R,2R)-*Cyclohexane*-1,2-*diyl*]*retronecine* (**4**) *and* 7,9-O,O-[(1S,2S)-*Cyclohexane*-1,2-*diyl*]*retronecine* (**5**).—(±)-*trans*-Cyclohexane-1,2,-dicarboxylic anhydride (100.6 mg, 0.653 mmol) was dissolved in a stirred solution of (+)-retronecine (94.3 mg, 0.608 mmol) in DME (10 ml) under argon. A colourless precipitate formed almost immediately. After the mixture had been stirred for 45 min, triphenylphosphine (318.7 mg, 1.215 mmol) and 2,2′-dithiodipyridine (267.7 mg, 1.215 mmol) were added and the mixture was stirred vigorously for 18 h. The clear yellow solution was then added by syringe during 20 min to DME (60 ml) heated at reflux under argon. After 14 h the solution was evaporated to dryness and partially purified by acid–base recycling as described for compound (**3**) above. The green oil so obtained was further purified by column chromatography on basic alumina with 15% v/v chloroform in dichloromethane as eluant. This afforded a mixture of compounds (**4**) and (**5**) as a colourless oil (25.1 mg, 14%) which could not be separated by additional column chromatography or by p.l.c. with a variety of solvent systems (R_F 0.62 in $CHCl_3$–MeOH–NH_3; 85:14:1); $[\alpha]_D^{21}$ + 55.0° (*c* 1.68 in $CHCl_3$); $\nu_{max.}$(CCl_4) 1 740 cm^{-1}; δ_H (200 MHz) 1.05—2.60 (13 H, complex, 6-H_2, 5β-H, and cyclohexane ring protons), 3.25 (1 H, m, 5α-H), 3.43 (1 H, m, 3β-H), 3.86 (1 H, m, 3α-H), 4.00 (1 H, ABq, *J* 12 Hz, 9-H), 4.31 (1 H, m, 8-H), 5.28 [1 H, ABq, *J* 12 Hz, 9-H of minor isomer (**5**)], 5.32 [1 H, ABq, *J* 12 Hz, 9-H of major isomer (**4**)]. 5.44 (1 H, m, 7-H), and 5.94 (1 H, br s, 2-H); δ_C (50 MHz) [Major isomer (**4**)] 24.05 (C-17 and -18), 27.48 and 27.53 (C-16 and -19), 34.4 (C-6), 46.3 and 46.7 (C-12 and -13), 54.1 (C-5), 60.2 (C-9), 61.3 (C-3), 74.0 and 77.2 (C-7 and -8), 131.8 (C-1), 133.6 (C-2), 173.7 and 174.0 (C-11 and -14); δ_C [minor isomer (**5**)] 20.7, 23.9, 24.7 and 26.8 (C-16, -17, -18, and -19), 34.3 (C-6), 41.5 and 47.4 (C-12 and -13), 53.9 (C-5), 60.5 (C-9), 61.0 (C-3), 74.5 and 76.9 (C-7 and -8), 132.1 (C-1), 133.3 (C-2), 172.6 and 174.0 (C-11 and -14). [The ratio of major isomer (**4**) to minor isomer (**5**) was 3.8:1]; *m/z* 291 (M^+) (27%), 138, 137, 136, 120, 119, 94, 93 (100%), and 80 (Found: M^+, 291.1467. $C_{16}H_{21}NO_4$ requires *M* 291.1470).

(1R,2R)-(−)-*Cyclohexane*-1,2-*dicarboxylic acid.*—A solution of the mixture of macrocyclic dilactones (**4**) and (**5**) (12.22 mg, 0.0420 mmol), obtained from the previous experiment, and barium hydroxide octahydrate (54.7 mg, 0.173 mmol) in water

(20 ml) was heated at reflux for 2 h. The solution was then acidified to pH 1 with conc. hydrochloric acid and continuously extracted with diethyl ether (30 ml) for 60 h. After the ethereal extracts had been evaporated to dryness, the residue was dissolved in acetone (5 ml) which was then dried, filtered, and concentrated to yield *trans*-cyclohexane-1,2-dicarboxylic acid as a colourless amorphous solid (7.16 mg, 99%) which exhibited i.r. and n.m.r. spectra identical with those of the racemic compound; $[\alpha]_D^{21}$ − 12.29° (*c* 0.716 in acetone) {lit.,[13] $[\alpha]_D^{30}$ + 22.3° (*c* 5.3 in acetone) for the (1*S*,2*S*)-(+)-enantiomer}. This gave an optical purity of *ca.* 55% for the (1*R*),(2*R*)-(−)-cyclohexane-1,2-dicarboxylic acid and therefore established structure (**4**) as the major isomer and (**5**) as the minor isomer in 3.45:1 ratio.

(+)-7,9-O,O-(*Phthaloyl*)*retronecine* (**6**).—Phthalic anhydride (87.1 mg, 0.588 mmol) was dissolved in a solution of (+)-retronecine (86.9 mg, 0.560 mmol) in DMF (10 ml) with stirring under argon. After *ca.* 5 min a colourless precipitate formed and stirring was continued for 1 h. Triphenylphosphine (220 mg, 0.840 mmol) and 2,2′-dithiodipyridine (185 mg, 0.840 mmol) were added and brisk stirring was continued for 3 days until a homogeneous yellow solution was obtained. This solution was diluted with DMF (50 ml) and heated at 140 °C for 3 h under argon. The mixture was cooled and concentrated, and the resulting green residue was dissolved in chloroform and subjected to acid–base recycling according to the procedure used with compound (**3**) above. The green oil thus obtained was purified by column chromatography on basic alumina and elution with 10% v/v chloroform in dichloromethane. The macrocyclic diester (**6**) was collected as a colourless oil (25 mg, 16%) which crystallised on standing. 7,9-O,O-(*Phthaloyl*)*retronecine* was obtained as needles, m.p. 145—146 °C (hexane); $[\alpha]_D^{19}$ + 239° (*c* 0.074 in $CHCl_3$); R_F 0.65; $\nu_{max.}(CCl_4)$ 1 725 cm^{-1}; δ_H (360 MHz) 2.02 and 2.17 (2 H, m, 6-H_2), 2.64 (1 H, m, 5β-H), 3.20 (1 H, m, 5α-H), 3.49 (1 H, m, 3β-H), 3.87 (1 H, m, 3α-H), 4.27 (1 H, ABq, *J* 12 Hz, 9-H), 4.50 (1 H, m, 8-H), 5.55 (1 H, ABq, *J* 12 Hz, 9-H), 5.80 (1 H, m, 7-H), 6.08 (1 H, br s, 2-H), 7.56 (2 H, m, 17- and 18-H), and 7.76 (2 H, m, 16- and 19-H); δ_C (90 MHz) 33.6 (C-6), 53.7 (C-5), 60.6 (C-9), 61.3 (C-3), 75.5 and 77.4 (C-7 and -8), 129.6 (C-17 and -18), 131.4 and 131.6 (C-16 and -19), 131.8, 132.1, and 132.6 (C-1, -12, and -13), 135.4 (C-2), and 167.7 and 167.8 (C-11 and -14); *m/z* 285 (M^+) (21%), 138, 137, 136, 120, 119, 94, 93, 84 (100%), and 80 (Found: M^+, 285.1003. $C_{16}H_{15}NO_4$ requires *M*, 285.1001) (Found: C,67.6; H, 5.2; N, 5.0. $C_{16}H_{15}NO_4$ requires C, 67.4; H, 5.3; N, 4.9%).

Acknowledgements

We are grateful to the S.E.R.C. for a Research Assistantship (to M. B.).

References

1 L.B. Bull, C. C. J. Culvenor, and A. T. Dick, 'The Pyrrolizidine Alkaloids,' North-Holland, Amsterdam, 1968.
2 D. J. Robins, *Fortschr. Chem. Org. Naturst.*, 1982, **41**, 115; 'The Alkaloids,' Specialist Periodical Reports, The Royal Society of Chemistry, London, 1971—1983, vols. 1—13.
3 A. R. Mattocks, 'Phytochemical Ecology,' ed. J. B. Harborne, Academic Press, London and New York, 1972, p. 179.
4 A. R. Mattocks, *Toxicol. Lett.*, 1982, **14**, 111.
5 K. Brown, J. A. Devlin, and D. J. Robins, *J. Chem. Soc., Perkin Trans. 1*, 1983, 1819; J. A. Devlin and D. J. Robins, *J. Chem. Soc., Chem. Commun.*, 1981, 1272.
6 K. Narasaka, T. Sakakura, T. Uchimaru, K. Morimoto, and T. Mukaiyama, *Chem. Lett.*, 1982, 445.
7 E. Vedejs and S. D. Larsen, *J. Am. Chem. Soc.*, 1984, **106**, 3030.
8 J. Huang and J. Meinwald, *J. Am. Chem. Soc.*, 1981, **103**, 861.
9 J. A. Devlin, D. J. Robins, and S. Sakdarat, *J. Chem. Soc., Perkin Trans. 1*, 1982, 1117; D. J. Robins and S. Sakdarat, *J. Chem. Soc., Chem. Commun.*, 1980, 282.
10 D. H. G. Crout, N. M. Davies, E. H. Smith, and D. Whitehouse, *J. Chem. Soc., Perkin Trans. 1*, 1972, 671.
11 D. J. Robins and J. R. Sweeney, *J. Chem. Soc., Perkin Trans. 1*, 1981, 3083.
12 E. J. Corey and K. C. Nicolaou, *J. Am. Chem. Soc.*, 1974, **96**, 5614.
13 D. E. Applequist and N. D. Werner, *J. Org. Chem.*, 1963, **28**, 48.
14 H. J. Huizing, F. DeBoer, and T. M. Malingré, *J. Chromatogr.*, 1980, **195**, 407; R. J. Molyneux and J. N. Roitman, *ibid.*, p. 142.
15 D. R. Burfield and R. H. Smithers, *J. Org. Chem.*, 1978, **43**, 3966.

Received 11*th July* 1984; *Paper* 4/1199