Pyrrolizidine Alkaloid Analogues. Preparation of Semisynthetic Esters of Retronecine

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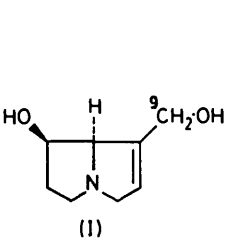
Methods have been devised for the selective esterification of retronecine (I) to give analogues of the naturally occurring hepatotoxic pyrrolizidine alkaloids. Selective esterification at C-9 of retronecine (I) with simple acids was achieved by using *NN'*-dicyclohexylcarbodi-imide. Selective esterification and esterification with αβ-unsaturated and α-hydroxy-αα-dialkyl acids was achieved through the intermediacy of *N*-acylimidazoles.

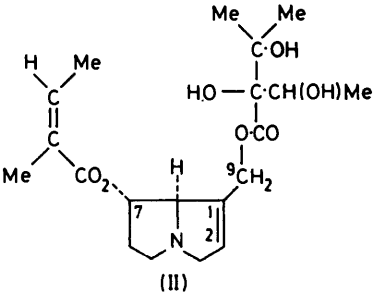
THE pyrrolizidine alkaloids have attracted much attention because of the specific hepatotoxicity and carcinogenicity exhibited by many members of the series.[1-4] The structural features necessary for toxicity have been defined.[2] As exemplified by the typical toxic alkaloid heliosupine (II), these are (i) the presence of a 1,2-double bond in the pyrrolizidine nucleus, and (ii) esterification at C-9. Toxicity is strongly dependent on the nature of the esterifying acid, and increases with the degree of steric hindrance at the α-carbon atom. Often, the esterifying (' necic ') acids either bear an α-hydroxy group or contain an αβ-double bond. Toxicity is enhanced by esterification at C-7. When both the C-7 and C-9 hydroxy groups are acylated the esterifying acids are usually different. Certain alkaloids occur as macrocyclic diesters of dihydroxy-1-methylpyrrolizidines and dicarboxylic acids.

Two general approaches to the synthesis of naturally occurring ester alkaloids have been developed. Culvenor and his co-workers prepared several monoester alkaloids from the 1-chloromethyl derivative of the hydroxypyrrolizidine (necine) and the sodium salt of the corresponding acid.[5] Kochetkov and his co-workers used transesterification in order to prepare naturally occurring esters of 1-hydroxymethylpyrrolizidines.[6] Semisynthetic diesters of retronecine have been prepared by Mattocks from the necine and various acid chlorides.[7] Other simple monoesters have been prepared by conventional methods.[8-10]

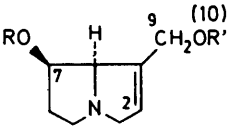
In order to investigate structure–activity relationships in this series and to discover procedures which would be applicable to the total synthesis of pyrrolizidine alkaloids, we wished to develop methods for the synthesis of diester alkaloids and analogues by the direct esterification of readily available necine bases such as retronecine (I). The initial requirements therefore were for methods for (i) selective esterification of dihydroxynecine bases such as retronecine (I) which would permit the synthesis of unsymmetrical diester alkaloids [*cf.* (II)], and (ii) esterification of necine bases with αβ-unsaturated and hindered α-hydroxy acids.



(I)



(II)



(III) R = EtO·CO·CH_2·CO, R' = H
(IV) R = H, R' = EtO·CO·CH_2·CO
(V) R = R' = EtO·CO·CH_2·CO
(VI) R = EtO·CO·$[CH_2]_3$·CO, R' = H
(VII) R = H, R' = EtO·CO·$[CH_2]_3$·CO
(VIII) R = H, R' = Cl_3C·CH_2·O·CO·$[CH_2]_3$·CO
(IX) R = Bu^tO·CO·$[CH_2]_3$·CO, R' = H
(X) R = H, R' = Bu^tO·CO·$[CH_2]_3$·CO
(XI) R = H, R' = MeCH:CMe·CO [(*Z*) and (*E*)]
(XIV) R = R' = (*E*)—MeCH:CMe·CO
(XVI) R = H, R' = (*E*)—MeCH:CMe·CO
(XVII) R = H, R' = Me_3C·CO
(XVIII) R = H R' = Me_2CH·CO
(XIX) R = H, R = $MeCH_2$·CO
(XX) R = Me_2CH·CO, R' = Me_3C·CO
(XXI) R = R' = $Me_2C(OH)$·CO

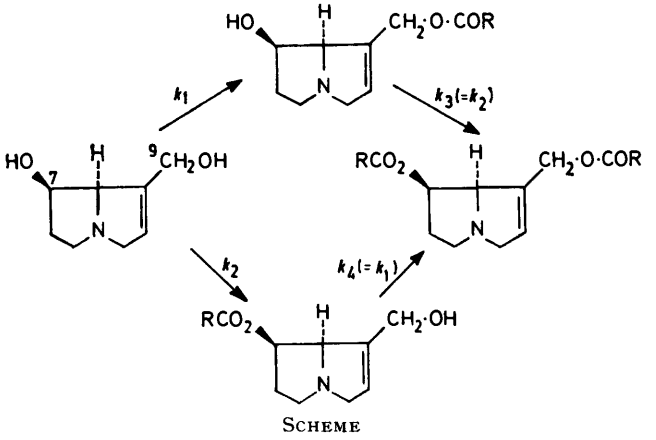
Specificity of esterification has been examined by using two methods not hitherto applied in this series: carbodi-imide coupling[11] and esterification by using acylimidazoles.[12] The results have been compared with those of esterification by acid chlorides.

Esterification of retronecine with an equimolar amount

[1] L. B. Bull, C. C. J. Culvenor, and A. T. Dick, ' The Pyrrolizidine Alkaloids,' North-Holland, Amsterdam, 1968.
[2] A. R. Mattocks, ' Phytochemical Ecology,' ed. J. B. Harborne, Academic Press, London and New York, 1972, p. 179.
[3] J. R. Allan, I. C. Hsu, and L. A. Carstens, *Cancer Res.*, 1975, **35**, 997.
[4] R. Schoental, *Cancer Res.*, 1975, **35**, 2020; *Biochem. Soc. Trans.*, 1975, **3**, 292.
[5] C. C. J. Culvenor, A. T. Dann, and L. W. Smith, *Chem. and Ind.*, 1959, 20; C. C. J. Culvenor and L. W. Smith, *Austral. J. Chem.*, 1966, **19**, 1955; C. C. J. Culvenor, S. R. Johns, J. A. Lamberton, and L. W. Smith, *ibid.*, 1970, **23**, 1279.
[6] N. K. Kochetkov, A. M. Likhosherstov, and A. S. Lebedeva, *Zhur. obshchei Khim.*, 1961, **31**, 3461.
[7] A. R. Mattocks, *J. Chem. Soc.* (*C*), 1969, 2698.
[8] N. K. Hart and J. A. Lamberton, *Austral. J. Chem.*, 1966, **19**, 1259.
[9] B. Lindström and B. Lüning, *Acta Chem. Scand.*, 1969, **23**, 3352.
[10] H. Tanino, S. Inoue, K. Nishikawa, and Y. Hirata, *Tetrahedron*, 1969, **25**, 3033.
[11] A. Buzas, C. Egnell, and P. Fréon, *Compt. rend.*, 1962, **255**, 945; F. Kurzer and K. Douraghi-Zadeh, *Chem. Rev.*, 1967, **67**, 107.
[12] H. A. Staab, *Angew. Chem. Internat. Edn.*, 1962, **1**, 351.

of a carboxylic acid can give any of three products: 9-monoester, 7-monoester, and 7,9-diester [*cf.* (II)]. All methods were expected to favour esterification at the C-9 primary allylic alcohol function rather than the C-7 secondary hydroxy group. However, lack of specificity would lead not only to the appearance of 7-monoester in the product mixture but also to appreciable quantities of diester. This can be seen from the simplified kinetic scheme shown.

If as a first approximation it is assumed that the rate of esterification at a given hydroxy group is the same regardless of whether or not the other hydroxy group is esterified, two rate constants, k_1 (= k_4) and k_2 (= k_3), suffice to cover the four steps of the Scheme. Specific esterification at C-9 will result when k_1 is finite and k_2 is zero. When k_2 is non-zero but small in comparison with k_1, 7-monoester will be formed but rapidly transformed into 7,9-diester because of the relatively large rate constant ($k_4 = k_1$) for step (iv). By contrast, 9-monoester will be transformed relatively slowly into diester since the corresponding reaction (iii) is governed by the relatively small rate constant k_3 (= k_2). This greatly simplified treatment shows that the criterion of specificity must be satisfied not only in the formation of



SCHEME

minimum quantities of 7-monoester but also in the formation of minimum quantities of diester.

Initial esterifications were carried out with derivatives of dibasic acids considered to be potential intermediates for the synthesis of macrocyclic diester analogues.

Treatment of retronecine with an equimolar amount of ethyl hydrogen malonate in chloroform in the presence of *NN'*-dicyclohexylcarbodi-imide gave a mixture of monoesters and diester in the ratio 80 : 20 and in an overall yield of 60%. The proportion of 7- (III) to 9-monoester (IV) (1 : 2) was determined from the 100 MHz n.m.r. spectrum of the mixture. Acylation at C-7 and C-9 results in the appearance of characteristic signals for the C-7 methine and the C-9 methylene protons.[13] The ratio of 7- to 9-monoester determined from the integrations of these signals was confirmed by the integrations of the signals due to the C-2 vinyl protons, which appear with different chemical shifts in the spectra of the two monoesters. These criteria were used in the analysis of all subsequent product mixtures. The signals due to the C-9 monoester (IV) appeared as an AB quartet (J 12 Hz). Non-equivalence of the C-9 protons in open-chain esters of retronecine has been noted previously only in the spectra of those alkaloids with bulky α-substituents in the esterifying acid, indicating restricted rotation about the C(9)–O(10) bond.[14] The non-equivalence may arise through hydrogen bonding between the C-7 hydroxy group and the ethoxycarbonyl group as suggested for the comparable case of heliotrine.[15] Esterification of retronecine with a 2 : 1 molar ratio of ethyl hydrogen malonate to retronecine gave the diester (V) as an unstable gum from which no crystalline derivatives could be prepared. The mass spectra of these esters were in agreement with the spectra of alkaloids with comparable structures. The isomeric monoesters could not be separated in the usual chromatographic systems employed in this series.

Esterification of retronecine with 1 mol. equiv. of ethyl hydrogen glutarate under the same conditions gave a mixture of the 7- (VI) and 9- (VII) monoestérs in the ratio 2 : 7 and in an overall yield of 70%. No diester was detected by t.l.c. (Monoesters of retronecine have R_F values very different from those of the corresponding diesters in the solvent systems employed). Esterification with 1 mol. equiv. of 2,2,2-trichloroethyl hydrogen glutarate gave a 50% yield of 9-monoester (VIII) with no detectable 7-ester or diester. Under similar conditions t-butyl hydrogen glutarate gave a 1 : 5 mixture of the 7- (IX) and 9- (X) monoesters in 40% yield. From these results it was concluded that esterification by dicyclohexylcarbodi-imide provided a reasonably selective method for esterification of the C-9 hydroxy group of retronecine. It was significant that when equimolar amounts of retronecine and acid were used, appreciable amounts of diester were formed only with the least bulky acid, ethyl hydrogen malonate. This result is readily explicable since the presence of a sterically bulky ester substituent at either C-7 or C-9 would be expected to hinder esterification of the remaining hydroxy group. The approximations that $k_1 = k_4$ and $k_2 = k_3$ (Scheme) would therefore not hold under these conditions; these equalities would be replaced by the inequalities $k_4 < k_1$, $k_3 < k_2$.

Esterification by the di-imide method was next applied to the synthesis of esters of retronecine with an αβ-unsaturated acid, angelic acid. Initial experiments gave a low yield (30%) of C-9 monoester (XI), which gave an n.m.r. spectrum similar to that reported for 9-angelylretronecine.[5c] However, in addition to the quartet at τ 3.95 attributable to the vinyl proton of the angelyl residue, an additional quartet at τ 3.14 was observed which was attributable to the vinyl proton of a tiglyl

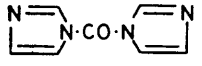
[13] C. C. J. Culvenor, M. L. Hefferman, and W. G. Woods, *Austral. J. Chem.*, 1965, **18**, 1605.

[14] C. C. J. Culvenor and W. G. Woods, *Austral. J. Chem.*, 1965, **18**, 1625.

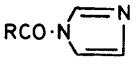
[15] Ref. 1, p. 53.

residue. It was concluded that during esterification, partial isomerisation of the angelyl residue to a tiglyl residue had occurred. The ratio of angelyl to tiglyl ester was 3 : 1, as determined by integration of the olefinic signals in the n.m.r. spectrum. The two-component nature of the mixture was confirmed by g.l.c., and assignment of the ester functions to C-9 was confirmed by the mass spectrum, which revealed a fragmentation pattern typical of C-9 monoesters, the most characteristic feature of which is loss of the allylic ester function to give an ion of *m/e* 138.[16,17] (C-7 Monoesters give a characteristic ion of *m/e* 137, whereas diesters give a major ion of *m/e* 136.[16-18]) The major product from the esterification was *N*-angelyldicyclohexylurea. The identity of this product was confirmed by direct synthesis from *NN'*-dicyclohexylcarbodi-imide and angelic acid. Various methods were investigated to suppress *N*-acylurea formation and to enhance the desired mode of esterification.[19,20] These included the addition to the reaction medium of pyridine hydrochloride, imidazole, and 1-hydroxybenzotriazole and the use of different solvents (pyridine, acetonitrile, dimethyl sulphoxide, and t-butyl alcohol). However, none of these modifications gave an increased yield of 9-angelylretronecine. The combination of low yield (due to competing rearrangement to *N*-acylurea) and partial geometrical isomerisation indicated that the carbodi-imide method was not suitable for the esterification of retronecine with αβ-unsaturated acids. Accordingly a different method of esterification was examined.

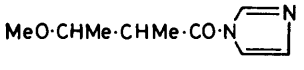
Staab and his co-workers have shown that through the intermediacy of acylimidazoles (azolides) (XIII) formed from *NN'*-carbonyldi-imidazole (XII) and carboxylic acids, esters can be produced on treatment of the reactive intermediates with alcohols.[12] This method appeared to be particularly well suited to the problem under investigation since it can be applied successfully to the synthesis of highly hindered esters. Treatment of retronecine in tetrahydrofuran with tiglic acid (2 mol. equiv.) and *NN'*-carbonyldi-imidazole with imidazolylsodium as catalyst gave a complex mixture of products



(XII)



(XIII)



(XV)

which after extensive fractionation gave (*E,E*)-7,9-bis-(2-methylbut-2-enoyl)retronecine (XIV) as an unstable, uncrystallisable gum (53%) together with a major crystalline product, $C_9H_{14}N_2O_2$, the n.m.r., i.r., and mass spectra of which indicated that it was *N*-(3-methoxy-2-methylbutanoyl)imidazole (XV). This compound was clearly formed by the addition of methanol, added during the work-up, to tiglylimidazole persisting in the reaction mixture. The adduct (XV) was similarly formed when tiglic acid and *NN'*-carbonyldi-imidazole in tetrahydrofuran were treated with methanol and a catalytic amount of imidazolylsodium, together with methyl tiglate These products were shown (g.l.c.) to be formed in approximately equal amounts. The apparent homogeneity of the imidazole derivative (XV) indicated that only one isomer was formed. When equimolar amounts of retronecine and tiglic acid in tetrahydrofuran were treated with *NN'*-carbonyldi-imidazole in the absence of catalyst, a 40% yield of crystalline 9-tiglylretronecine (XVI) was obtained. The structure was established on the basis of the elemental analysis, the n.m.r. spectrum, and comparison with the material prepared from 1-chloromethylretronecine hydrochloride and sodium tiglate by the method of Culvenor *et al.* The latter method, however, gave 9-tiglylretronecine in an overall yield of 20% based on retronecine.

Since the carbonyldi-imidazole method was successful when applied to the synthesis of 9-tiglylretronecine, further studies were undertaken with pre-prepared acylimidazoles. Thus treatment of retronecine with an equimolar amount of pivaloylimidazole for 24 h gave the crystalline 9-pivaloyl ester (XVII) of retronecine directly in 75% yield. The corresponding crystalline 9-isobutyryl (XVIII) and 9-propionyl (XIX) esters were prepared similarly in 60 and 40% yields respectively (Table). In these cases, increasing amounts of 7,9-diester were also formed. The lowering of specificity

Yields of C-9 monoesters on esterification of retronecine (I) by various procedures

	% Yield from use of		
Esterifying acid	*N*-Acylimidazole	*NN'*-Carbonyldi-imidazole	Acid chloride
Pivalic acid	75	40	35
Isobutyric acid	40	70	25
Propionic acid	40 [a]	50	20 [b]

[a] *Ca.* 25% diester formed. [b] More than 50% diester formed.

with decreasing steric size of the acyl component which was noted in the carbodi-imide couplings was thus paralleled in the acylimidazole procedure. The foregoing reactions were carried out in the absence of a catalyst. The reaction between pivaloylimidazole and retronecine in the presence of imidazolylsodium gave the 9-ester in a yield similar to that of the uncatalysed reaction, but with a shorter reaction time (2 h).

The simplicity and regiospecificity of the acylimidazole procedure made possible the achievement of a further objective, the synthesis of unsymmetrical diesters of retronecine. However, in attempting the esterification of 9-pivaloylretronecine with isobutyrylimidazole, the sensitivity of this reagent to the environment of the

[16] Ref. 1, p. 54.
[17] E. Pedersen and E. Larsen, *Org. Mass Spectrometry*, 1970, **4** (Suppl.), 249.
[18] T. Furuya and K. Araki, *Chem. and Pharm. Bull.* (*Japan*), 1968, **16**, 2521.
[19] C. H. Hassall, T. G. Martin, J. A. Schofield, and J. O. Thomas, *J. Chem. Soc.* (*C*), 1967, 997.
[20] Y. S. Klausner and M. Bodansky, *Synthesis*, 1972, 453.

hydroxy function to be esterified became a drawback rather than an asset and only slow conversion into the diester was observed. However, by using isobutyryl chloride in pyridine the diester (XX) was obtained in 50% yield as a gum which gave a crystalline picrate.

The acylimidazole method of esterification was compared with esterification of retronecine with 1 mol. equiv. of acid chloride in pyridine. Thus with isobutyryl chloride a 25% yield of 9-monoester (XVIII) was obtained with no detectable 7-ester. However, the major product was the 7,9-diester, indicating a much lower specificity than obtainable by the acylimidazole method. Similar results were obtained with propionyl chloride and pivaloyl chloride (Table).

With the demonstration that specific esterifications could be achieved with acylimidazoles, attention was again directed to direct synthesis by using the acid, the necine base, and *NN'*-carbonyldi-imidazole. It was found that the previously used solvent, tetrahydrofuran, gave poor results, a mixture of products being obtained. Similar results were obtained with benzene, dichloromethane, and acetone. However, in pure (ethanol-free) chloroform the yields of 9-monoester were comparable with those obtained by the direct use of acylimidazoles (Table). In addition, use of this solvent permitted the direct synthesis of 9-tiglylretronecine (XVI) from tiglic acid, retronecine, and *NN'*-carbonyldi-imidazole in 50% yield.

Finally, this method was applied to the synthesis of a diester of retronecine with an acid that displayed the characteristics most frequently associated with naturally occurring necic acids, *i.e.* with an acid having α-hydroxy-αα-dialkyl substitution. 2-Hydroxy-2-methylpropionic acid was chosen since, as it was achiral, problems associated with the formation of diastereoisomeric products would be avoided. Esterification of retronecine with 2 mol. equiv. of this acid in chloroform in the presence of *NN'*-carbonyldi-imidazole gave the corresponding 7,9-diester (XXI) of retronecine in 95% yield. The ester was characterised as the crystalline picrolonate.

Monoester alkaloids of α-hydroxy-αα-dialkylcarboxylic acids have been prepared by Culvenor *et al.*,[5] using the indirect method *via* 1-chloromethylpyrrolizidines and the sodium salt of the carboxylic acid, and by Kochetkov *et al.*[6] in low yield, by transesterification. However, the method demonstrated here offers a more direct and efficient route to alkaloids of this class.

EXPERIMENTAL

M.p.s are corrected. I.r. spectra were determined with a Hilger H900 Infrascan or Perkin-Elmer 357 spectrometer. Proton n.m.r. spectra were determined with a Perkin-Elmer R-60 or JEOL MH-100 spectrometer, for solutions in deuteriochloroform with tetramethylsilane as internal standard. Mass spectra were determined with a Perkin-Elmer–Hitachi RMU spectrometer with an electron beam energy of 80 eV. For chromatography, basic alumina (B.D.H.) was used. Neutral alumina was prepared by keeping neutral alumina (B.D.H) in ethyl acetate for 48 h with occasional stirring. The ethyl acetate was removed by filtration and the alumina was washed with methanol, dried in air, and activated at 110 °C for 16 h. T.l.c. was carried out on pre-coated Silica Gel PF_{254} plates (Merck) with the solvent system chloroform–methanol–ammonia (85 : 14 : 1). Pyrrolizidine derivatives were located with the modified Dragendorff reagent.[21] Analytical g.l.c. was performed on Pye Argon and Pye Series 104 chromatographs. Chloroform for esterifications was freed from ethanol by shaking five times with an equal volume of water, drying over calcium chloride in the dark for 24 h, and distilling. The purified chloroform was stored in the dark and used within 1 week. Tetrahydrofuran was boiled under reflux over copper(I) chloride and lithium aluminium hydride. Prior to use it was distilled from potassium.

Retronecine (I).—Monocrotaline (isolated from *Crotalaria spectabilis*, 20 g, 0.062 mol) and barium hydroxide octahydrate (39.5 g, 0.125 mol) were boiled under reflux in water (200 cm^3) for 2 h. The solution was cooled, treated with solid CO_2, filtered, acidified (Congo Red) with hydrochloric acid (1 mol dm^{-3}) and extracted continuously with ether for 24 h. The aqueous residue was passed through a column of Dowex 1-X8 ion-exchange resin (OH^- form; 100 g) and elution (H_2O) was continued until the eluate was neutral. The eluate was evaporated and extracted with three portions of boiling acetone. The acetone was evaporated off and the residue was crystallised (acetone) to give retronecine (I) (8.0 g, 85%) as large prisms, m.p. 120—121°.

Ethyl Hydrogen Malonate.—This was prepared as reported [22] and purified as the dicyclohexylamine salt, which crystallised from acetone–light petroleum (b.p. 60—80 °C) as needles, m.p. 113.5—114° (Found: C, 65.1; H, 10.0; N, 4.9. $C_{17}H_{31}NO_4$ requires C, 65.15; H, 10.0; H, 4.5%). $\nu_{max.}$ (KBr) 1 735 (ester C:O) and 1 640 cm^{-1} (CO_2^-). The free half ester was regenerated by extraction of an acidified aqueous solution of the salt with ether.

7,9-*Bis*-O-(2-*ethoxycarbonylacetyl*)*retronecine* (V).—Retronecine (200 mg, 1.29 mmol) and *NN'*-dicyclohexylcarbodi-imide (DCCI) (550 mg, 2.66 mmol) were stirred in chloroform (3 cm^3). Ethyl hydrogen malonate (340 mg, 2.60 mmol) in chloroform (1 cm^3) was added dropwise. The solution was stirred for 24 h and filtered, and the filtrate was extracted with dilute sulphuric acid (4 × 5 cm^3; 0.5 mol dm^{-3}). The extracts were combined, washed with chloroform (2 × 10 cm^3), neutralised with sodium hydroxide solution (0.1 mol dm^{-3}), brought to pH 8.2 with disodium hydrogen phosphate solution, and extracted with chloroform (5 × 15 cm^3). The extracts were combined, dried (Na_2SO_4), and evaporated to give a gum (300 mg), which appeared (t.l.c.) to consist of two components, R_F 0.25 and 0.65 (relative areas *ca.* 1 : 5). A portion (100 mg) of this material was purified by preparative t.l.c. on plates (20 × 20 cm) coated with Kieselgel PF_{254} (25 g). The major band was soaked in sulphuric acid (0.1 mol dm^{-3}) for 12 h. The extract was filtered, washed with chloroform (2 × 5 cm^3), neutralised with sodium hydroxide (0.1 mol dm^{-3}), brought to pH 8.2 with disodium hydrogen phosphate solution, and extracted with chloroform (4 × 10 cm^3). The combined extracts were dried (Na_2SO_4) and evaporated to give the diester (V) (20 mg) as a gum, from which a crystalline derivative could not be prepared; $\nu_{max.}$ ($CHCl_3$) 1 760—1 730 cm^{-1} (ester C:O), τ (100 MHz) 4.67 (1 H, m,

[21] R. Munier and M. Macheboef, *Bull. Soc. Chim. biol.*, 1951, **33**, 846.
[22] M. Freund, *Ber.*, 1884, **17**, 780.

7-H), 5.32br (2 H, s, 9-H), 5.82 (2 H, q, J 7 Hz, CH_2Me), 5.86 (2 H, q, J 7 Hz, CH_2Me), 6.65 (1 H, s, $CO{\cdot}CH_2{\cdot}CO_2$), 6.71 (2 H, s, $CO{\cdot}CH_2{\cdot}CO_2$), and 8.75 (6 H, t, J 7 Hz 2 × $MeCH_2$), *m/e* 383 (4%, M^+), 353 (6), 252 (14), 136 (20), 120 (40), 119 (29), 93 (100), and 80 (22).

7- (III) *and* 9-O-(2-*Ethoxycarbonylacetyl*)*retronecine* (IV).—Retronecine (100 mg, 0.64 mmol) was esterified with ethyl hydrogen malonate (85 mg, 0.64 mmol) and DCCI (140 mg, 0.68 mmol) as above, to give a product (90 mg), R_F 0.25 and 0.65. Preparative t.l.c. as before gave the diester (V) and a mixture of monoesters (III) and (IV). The ratio of diester to monoester was 1 : 4; τ [for the mixture of (III) and (IV)] 4.64 [m, 7-H (C-7 monoester)], 5.14 and 5.32 [ABq, J 12 Hz (C-9 monoester)]. From the integrations of these signals the ratio of the monesters (III) and (IV) was 1 : 2.

7- (VI) *and* 9-O-(4-*Ethoxycarbonylbutanoyl*)*retronecine* (VII).—To retronecine (I) (100 mg, 0.64 mmol) and DCCI (140 mg, 0.68 mmol) in chloroform (2 cm³) was added dropwise, with stirring, a solution of ethyl hydrogen glutarate [23] (103 mg, 0.64 mmol) in chloroform (0.5 cm³). The mixture was stirred for 3 h, filtered, and extracted with sulphuric acid (0.2 mol dm⁻³; 3 × 5 cm³). The combined extracts were washed with chloroform (2 × 5 cm³), brought to pH 8.5 with dilute ammonium hydroxide, and extracted with chloroform (4 × 10 cm³). The combined extracts were dried (Na_2SO_4) and evaporated to give the mixture of monoesters (VI) and (VII) as a gum (130 mg), R_F 0.17, $\nu_{max.}$ ($CHCl_3$) 3 580 (OH) and 1 735 cm⁻¹ (ester C:O), τ 4.73 [m, 7-H (C-7 ester)] and 5.32 [s, 9-H_2 (C-9 ester)], *m/e* 297 (9%, M^+), 163 (28), 155 (21), 138 (44), 137 (15), 111 (24), 94 (31), 93 (100), 83 (57), 82 (30), 81 (52), and 80 (46).

9-O-[4-(2,2,2-*Trichloroethoxycarbonyl*)*butanoyl*]*retronecine* (VIII).—Retronecine (I) (100 mg, 0.64 mmol) was esterified with 2,2,2-trichloroethyl hydrogen glutarate [24] (170 mg, 0.64 mmol) in the presence of DCCI (140 mg, 0.68 mmol) as above to give the monoester (VIII) as a gum (130 mg), R_F 0.23, $\nu_{max.}$ ($CHCl_3$) 3 570 (OH), 3 480 (hydrogen-bonded OH), and 1 735 cm⁻¹ (ester C:O), τ 5.24 (2 H, s, 9-H_2), *m/e* 405 (1%, M + 6), 403 (2, M + 4), 401 (5, M + 2), 399 (5, M^+), 155 (42), 138 (100), 137 (55), 136 (35), 111 (28), 94 (73), 93 (100), and 80 (55).

9-(X) *and* 7-O-(4-*t-Butoxycarbonylbutanoyl*)*retronecine* (IX).—Retronecine (I) (100 mg, 0.64 mmol) was esterified with t-butyl hydrogen glutarate [25] (120 mg, 0.64 mmol) in the presence of DCCI (140 mg, 0.68 mmol) as above, to give a mixture of the monoesters (IX) and (X) (80 mg) as a gum, R_F 0.20, τ 4.68 [m, 7-H (C-7 ester)] and 5.29 [s, 9-H_2 (C-9 ester)], in the ratio 1 : 5 (n.m.r.), *m/e* 325 (3%, M^+), 155 (16), 154 (25), 138 (62), 137 (34), 136 (31), 115 (75), 93 (100), 87 (27), and 80 (37).

(Z)- *and* (E)-9-O-(2-*Methylbut-2-enoyl*)*retronecine* (XI).—Retronecine (100 mg, 0.64 mmol) was esterified with angelic acid (64 mg, 0.64 mmol) in the presence of DCCI (140 mg, 0.68 mmol) for 4 days. The product was isolated as before to give a mixture of the monoesters (XI) as a gum (20 mg), R_F 0.25, τ 3.13 [q, J 7 Hz, (*Z*)-MeC*H*:], 3.90 [q, J 7 Hz, (*E*)-MeC*H*:], and 5.25 (s, 9-H), in the ratio [(*E*)- to (*Z*)-ester] of 1 : 3 (n.m.r.), *m/e* 237 (4%, M^+), 184 (14), 138 (27), 137 (25), 93 (100), and 80 (17). G.l.c. [Pye Argon; column 4 ft × ⅜ in; 3% SE 30 on Chromosorb W (80—100 mesh); column temperature 190 °C; gas flow rate 60 cm³ min⁻¹] retention times were: (*Z*)-ester 6.88; (*E*)-ester 8.44 min. Resolution and integration of the peaks (Dupont 310 Curve Resolver) gave a ratio of (*E*)- to (*Z*)-ester of *ca.* 1 : 4.

(Z)-NN′-*Dicyclohexyl*-N-(2-*methylbut-2-enoyl*)*urea*.—(*a*) The chloroform residue and the chloroform washings from the previous experiment were combined, dried (Na_2SO_4), and evaporated to give a gum (150 mg), R_F 0.69 and 0.75. The two components were separated by preparative t.l.c. (Kieselgel PF_{254}), with methanol as eluant to give *NN′*-dicyclohexylurea (15 mg), m.p. 224—225°, R_F 0.69, and the (*Z*)-*methylbutenoyl derivative* (100 mg), R_F 0.75, m.p. 118—119° [from light petroleum (b.p. 60—80 °C)] (Found: C, 70.85; H, 10.05; N, 9.3. $C_{18}H_{30}N_2O_2$ requires C, 70.55; H, 9.9; N, 9.15%), $\nu_{max.}$ (KBr) 3 440 and 3 340 (NH), 1 680 (amide I), 1 645 (tert. amide), and 1 530 cm⁻¹ (amide II), τ 1.8br (1 H, s, NH), 4.64 (1 H, q, J 7 Hz, MeC*H*), 6.04—6.50 (2 H, m, N·CH), 8.14 (3 H, s, :C*Me*·CO_2), and 8.37 (3 H, d, J 7 Hz, *Me*CH:).

(*b*) To a boiling solution of DCCI (1.0 g, 4.8 mmol) in pyridine (20 cm³) was added dropwise a solution of angelic acid (480 mg, 4.8 mmol) in pyridine (5 cm³). The solution was boiled under reflux for a further 30 min and cooled. The pyridine was evaporated off, the residue was dissolved in hot acetone, and the solution was filtered and evaporated to give a white solid, R_F 0.13 and 0.30 (Kieselgel G; $CHCl_3$). Part of the mixture (100 mg) was purified by preparative t.l.c. (Kieselgel PF_{254}). Elution of the major band gave the angelyl derivative (40 mg), m.p. 118—119°, R_F 0.30, identical (n.m.r. and i.r. spectra) with that obtained in (*a*).

Modifications of the Esterification Procedure.—Retronecine (5 mg, 0.032 mmol) and DCCI (6.9 mg, 0.033 mmol) were stirred in the solvent plus additive (total volume 0.4 cm³) and angelic acid (3.2 mg, 0.032 mmol) in the solvent (0.1 cm³) was added dropwise. The reaction was followed by g.l.c. as before. The following additives were used in chloroform solution (mole % with respect to DCCI; temperature 25 °C unless otherwise specified): pyridine hydrochloride (50%, 100%), imidazole (100%; −10 °C), 1-hydroxybenzotriazole (100%), pyridine (200%; −10 °C). The following solvents were used without additives: pyridine, dimethyl sulphoxide, chloroform (−10 °C), acetonitrile (−10 °C), t-butyl alcohol. In no case was the yield greater than in chloroform alone at 25 °C.

(E,E)-7,9-*Bis*-O-(2-*methylbut-2-enoyl*)*retronecine* (XIV).—*NN′*-Carbonyldi-imidazole (CDI) (250 mg, 1.54 mmol) and tiglic acid (130 mg, 1.30 mmol) were stirred in tetrahydrofuran (3 cm³) under dry nitrogen for 2 h. Retronecine (100 mg, 0.64 mmol) and imidazolylsodium in tetrahydrofuran (1 mol dm⁻³; 0.1 cm³) were added and the mixture was stirred for 20 h. The solvent was evaporated off at room temperature and the residue was chromatographed on Bio Glass Beads 2 500 (Bio-Rad Ltd.; 100 cm³) impregnated with phosphate buffer, pH 8.0. Elution with light petroleum (b.p. 60—80 °C) (10 cm³ fractions) gave, from fractions 5—35, the diester (XIV) (110 mg) as a gum, which was further purified by preparative t.l.c. [Kieselgel PF_{254} (Merck)]. The diester was obtained as a gum which gave a single peak on g.l.c. (160 °C; t_R 27.50 min) with a trace

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[24] D. S. Drain and J. G. B. Howes, B.P. 1,062,460 (*Chem. Abs.*, 1967, **67**, 2779t).

[25] A. L. McCloskey, G. S. Fonken, R. W. Kluiber, and W. S. Johnson, *Org. Synth.*, 1954, **34**, 26; H. J. Backer and J. D. H. Homan, *Rec. Trav. chim.*, 1939, **58**, 1048; J. D. H. Homan, *ibid.*, 1944, **63**, 189.

impurity, t_R 13.10 min; τ ($CDCl_3$) 3.24 (2 × 1 H, q, *J ca.* 7 Hz 2 × MeC*H*:), 4.22 (1 H, s, 2-H), 4.64 (1 H, m, 7-H), 5.32 (2 H, s, 9-H), 8.20 (6 H, d, *J ca.* 7 Hz 2 × *Me*CH:), 8.24 [6 H, m, MeC(CO_2):], *m/e* 319 (M^+, 10%), 237 (14), 220 (17), 219 (17), 136 (80), 120 (40), 119 (42), 93 (100), 83 (80), and 80 (20). Fractions 140—210 gave N-(3-*methoxy*-2-*methylbutanoyl*)*imidazole* (XV) as needles (40 mg) [from light petroleum (b.p. 60—80 °C)], m.p. 63.5—64° (Found: C, 59.55; H, 7.65; N, 15.4. $C_9H_{14}N_2O_2$ requires C 59.3; H, 7.7; N, 15.4%), $\nu_{max.}$ (KBr) 1 735 cm^{-1} (amide CO), τ ($CDCl_3$) 2.48, 2.91, and 3.07 (each 1 H, imidazole H), 5.61 [1 H, dq, *J* 7 and 7 Hz, MeC*H*(OMe)], 6.27 (3 H, s, O*Me*), 7.26 (1 H, dq, *J* 7 and 7 Hz, MeC*H*), 8.5 [3 H, d, *J* 7 Hz, *Me*C(OMe)], and 9.02 (3 H, d, *J* 7 Hz, *Me*CH), *m/e* 182 (M^+, 64%), 151 (13), 95 (100), 70 (21), 69 (23), 68 (52), and 59 (53).

Synthesis of N-(3-*Methoxy*-2-*methylbutanoyl*)*imidazole* (XV).—Methyl tiglate was prepared by a published procedure;[26] b.p. 136—138°. A solution of CDI (1.1 g, 6.8 mmol) and tiglic acid (0.52 g, 5.2 mmol) in tetrahydrofuran (10 cm^3) was stirred under dry nitrogen for 1 h. Methanol (170 mg, 5.2 mmol) and imidazolylsodium in tetrahydrofuran (1 mol dm^{-3}; 0.1 cm^3) were added and the mixture was stirred for a further 24 h. The product was examined by temperature-programmed g.l.c. [5 ft × 3/8 in glass column; 3% SE 30 on Chromosorb W (80—100 mesh); gas flow rate 40 cm^3 min^{-1}; temperature programme 50 °C (5 min), 50—110 °C (2° min^{-1}); 110 °C (10 min)]. Methyl tiglate and the derivative (XV) were identified by their retention times (3.5 and 39.5 min, respectively). Integration of the peaks showed that the two products were formed in approximately equal amounts. Methyl tiglate was isolated by preparative g.l.c. (column 20 ft × $\frac{5}{8}$ in; 30% SE 30 on 30—60 mesh Chromosorb W; column temperature 168 °C; gas flow rate 200 cm^3 min^{-1}). The n.m.r. spectrum was identical with that of authentic ester. The derivative (XV) was isolated by preparative t.l.c. [Kieselgel PF_{254} (Merck)] to give needles, m.p. 63.5—64° (from light petroleum).

(E)-9-O-(2-*Methylbut*-2-*enoyl*)*retronecine* (XVI).—(*a*) *By using CDI.* CDI (110 mg, 0.68 mmol) and tiglic acid (64 mg, 0.64 mmol) were stirred in tetrahydrofuran (2 cm^3) for 1 h under dry nitrogen. Retronecine (100 mg, 0.64 mmol) was added and the mixture was stirred for 24 h. The solvent was evaporated off, the residue was dissolved in sulphuric acid (0.2 mol dm^{-3}; 15 cm^3), and the acidic solution was washed with chloroform (3 × 5 cm^3), brought to pH 8.5 with ammonium hydroxide (2 mol dm^{-3}), and extracted with chloroform (4 × 10 cm^3). The extracts were dried (Na_2SO_4) and evaporated to give a gum which was applied in benzene to a column of neutral alumina (Grade I; 8 g). Elution with benzene gave minor compounds; elution with benzene–chloroform (4 : 1) gave the *monoester* (XVI) as a gum (60 mg), which crystallised [from light petroleum (b.p. 40—60 °C)] as needles, m.p. 64—65° (Found: C, 66.2; H, 8.35; N, 5.8. $C_{13}H_{19}NO_3$ requires C, 65.8; H, 8.1; N, 5.9%), $\nu_{max.}$ (KBr) 3 440—3 380 (OH), 1 710 (αβ-unsaturated ester C:O), 1 645 (conj. C:C), and 1 250 cm^{-1}, τ ($CDCl_3$) 3.12 (1 H, q, *J* 7 Hz, MeC*H*:), 4.15 (1 H, s, 2-H), 5.24 (2 H, s, 9-H_2), 8.15 [3 H, s, *Me*C(CO_2):], and 8.18 (3 H, d, *J* 7 Hz *Me*CH:), *m/e* 237 (M^+, 14%), 193 (22), 154 (54), 138 (86), 137 (90), 136 (41), 94 (74), and 93 (100).

[26] R. E. Buckles and G. V. Mock, *J. Org. Chem.*, 1960, **15**, 680.

(*b*) *From* 1-*chloromethylretronecine hydrochloride.*—A solution of 1-chloromethylretronecine [27] (250 mg, 1.19 mmol) and sodium tiglate (142 mg, 1.19 mmol) in 50% ethanol–water (5 cm^3) was boiled under reflux for 3 h. The ethanol was evaporated off under reduced pressure and the aqueous residue was brought to pH 8.5 with ammonium hydroxide (2 mol dm^{-3}) and extracted with chloroform (4 × 10 cm^3). The extracts were dried (Na_2SO_4) and evaporated to give the monoester (XVI) (110 mg).

Synthesis of C-9 *Monoesters of Retronecine from Acylimidazoles. General Procedure.*—Retronecine (100 mg, 0.64 mmol) and the *N*-acylimidazole [12] (0.64 mmol) were stirred in tetrahydrofuran (2—3 cm^3) for 24 h. The solvent was evaporated off and the residue was dissolved in chloroform (5 cm^3) and extracted with sulphuric acid (0.2 mol dm^{-3}; 4 × 5 cm^3). The extracts were combined, washed with chloroform (3 × 5 cm^3), brought to pH 8.5 with ammonium hydroxide (2 mol dm^{-3}), and extracted with chloroform (4 × 10 cm^3). The chloroform extracts were dried (Na_2SO_4) and evaporated. The product was purified by chromatography on neutral alumina. Minor impurities were eluted with benzene; the C-9 monoesters of retronecine were eluted with benzene–chloroform (4 : 1). By this method were obtained (yields given in the Table): 9-O-(2,2-*dimethylpropionyl*)*retronecine* (XVII), needles [from light petroleum (b.p. 60—80 °C)], m.p. 97—98° (Found: C, 65.3; H, 9.15; N, 5.7. $C_{13}H_{21}NO_3$ requires C, 65.25; H, 8.85; N, 5.85%), $\nu_{max.}$ (KBr) 3 490 (OH) and 1 725 cm^{-1} (ester C:O), τ 3.93 (1 H, s, 2-H), 5.13 (2 H, s, 9-H), and 8.72 (9 H, s, Me_3C), *m/e* 239 (M^+, 5%), 195 (8), 154 (7), 138 (54), 137 (19), 94 (34), and 93 (100); 9-O-(2-*methylpropionyl*)-*retronecine* (XVIII), needles [from light petroleum (b.p. 60—80 °C)], m.p. 62–63° (Found: C, 64.3; H, 9.0; N, 6.3. $C_{12}H_{19}NO_3$ requires C, 64.0; H, 8.5; N, 6.2%), $\nu_{max.}$ (KBr) 3 440 (OH) and 1 730 cm^{-1} (ester C:O), τ 4.15 (1 H, s, 2-H), 5.30 (2 H, s, 9-H), 7.40 (1 H, m, Me_2CH), and 8.84 (6 H, d, *J* 7 Hz, Me_2C), *m/e* 225 (M^+, 6%) 181 (12), 155 (15), 154 (6), 138 (51), 137 (29), 94 (41), and 93 (100); 9-O-*propionylretronecine* (XIX), needles [from light petroleum (b.p. 40—60°)], m.p. 58—59° (Found: C, 62.8; H, 8.5; N, 6.7. $C_{11}H_{17}NO_3$ requires C, 62.55; H, 8.1; N, 6.6%), $\nu_{max.}$ (KBr) 3 450 (OH) and 1 730 cm^{-1} (ester C:O), τ 4.13 (1 H, s, 2-H), 5.27 (2 H, s, 9-H), 7.61 (2 H, q, *J* 7 Hz, MeCH_2), and 8.83 (3 H, t, *J* 7 Hz, *Me*CH_2), *m/e* 211 (M^+, 7%), 167 (15), 155 (15), 138 (40), 137 (31), 130 (18), 94 (44), and 93 (100).

Synthesis of C-9 *Monoesters of Retronecine from Acid Chlorides. General Procedure.*—Retronecine (100 mg, 0.64 mmol) and the acid chloride (0.64 mmol) were stirred in tetrahydrofuran (2 cm^3) with pyridine (1.0 mmol) for 4 h. The products were isolated and purified as above. The yields of C-9 monoesters are given in the Table.

Synthesis of C-9 *Monoesters of Retronecine from* NN′-*Carbonyldi-imidazole. General Procedure.*—NN′-Carbonyldi-imidazole (110 mg, 0.68 mmol) and the carboxylic acid (0.64 mmol) were stirred in chloroform (2 cm^3) until CO_2 evolution stopped. Retronecine (100 mg, 0.64 mmol) was added and the mixture was stirred for 24 h. The products were isolated and purified as above. The yields of C-9 monoesters obtained are given in the Table.

9-O-(2,2-*Dimethylpropionyl*)-7-(2-*methylpropionyl*)*retronecine* (XX).—Retronecine (100 mg, 0.64 mmol) and *N*-(2,2-dimethylpropionyl)imidazole (105 mg, 0.64 mmol)

[27] R. Adams and B. L. Van Duuren, *J. Amer. Chem. Soc.*, 1954, **76**, 6379.

were stirred in tetrahydrofuran for 2 h with imidazolylsodium [1 mol dm^{-3} (in tetrahydrofuran); 0.1 cm^3]. The solvent was evaporated off and the residue was extracted with sulphuric acid (0.2 mol dm^{-3}; 4 × 5 cm^3). The acidic solution was washed with chloroform (3 × 5 cm^3), brought to pH 8.5 with ammonium hydroxide (2 mol dm^{-3}), and extracted with chloroform (4 × 10 cm^3). The chloroform extracts were dried (Na_2SO_4) and evaporated. The product (140 mg), without further purification, was dissolved in tetrahydrofuran (2 cm^3) and treated with 2-methylpropionyl chloride (70 mg) and pyridine (100 mg). The mixture was stirred for 24 h and the product was isolated as above. The crude diester (160 mg) was purified by chromatography on neutral alumina (5 g). Elution with benzene gave the diester (XX) (100 mg, 50% based on retronecine) as a gum, $\nu_{max.}$ ($CHCl_3$) 1 725 cm^{-1} (2 × ester C:O), τ 4.25br (1 H, s, 2-H), 4.72 (1 H, m, 7-H), 5.38br (2 H, s, 9-H_2), 8.8 (9 H, s, Me_3C), and 8.90 (6 H, d, *J* 7 Hz, Me_2CH), *m/e* 309 (M^+, 8%), 208 (95), 136 (62), 120 (57), 119 (37), 94 (38), 93 (100), and 80 (22); *picrate* (from ethanol), yellow needles, m.p. 120—121° (Found: C, 51.4; H, 5.7; N, 10.4. $C_{23}H_{30}N_4O_{11}$ requires C, 51.3; H, 5.6; N, 10.4%).

7,9,-*Bis*-O-(2-*hydroxy*-2-*methylpropionyl*)*retronecine* (XXI).—*NN'*-Carbonyldi-imidazole (230 mg, 1.40 mmol) and 2-hydroxy-2-methylpropionic acid [28] (145 mg, 1.36 mmol) were stirred in chloroform (2 cm^3). When evolution of carbon dioxide stopped, retronecine (100 mg, 0.64 mmol) was added and the mixture was stirred for 48 h. The diester was isolated as above, as a gum (200 mg) which gave a crystalline *picrolonate* (from ethanol), m.p. 155—156° (Found: C, 52.6; H, 5.8; N, 11.8. $C_{26}H_{33}N_5O_{11}$ requires C, 52.8; H, 5.6; N, 11.8%). The free base (XXI) was recovered by passing the picrolonate through a column of basic alumina (Grade I) in chloroform to give a gum, $\nu_{max.}$ ($CHCl_3$) 3 540 (OH) and 1 730 cm^{-1} (ester C:O), τ 4.19br (1 H, s, 2-H), 4.61 (1 H, m, 7-H), 5.29br (2 H, s, 9-H), 8.58 (6 H, s, 2 × Me), 8.63 (3 H, s, Me), and 8.67 (3 H, s, Me).

We thank the M.R.C. for a research studentship (to W. M. H.).

[6/1394 *Received*, 16*th July*, 1976]

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