Pergamon

Tetrahedron Vol. 50, No. 3, pp. 895-906, 1994

0040-4020/94 $6.00+0.00

0040-4020(93)E0058-N

X=Y-ZH Compounds as Potential 1,3-Dipoles. Part 41.[1] Azomethine Ylide Formation from the Reactions of α-Amino Acids and Esters with Alloxan (Strecker Degradation) and with 1-Phenyl-3-methylpyrazolin-4,5-dione.

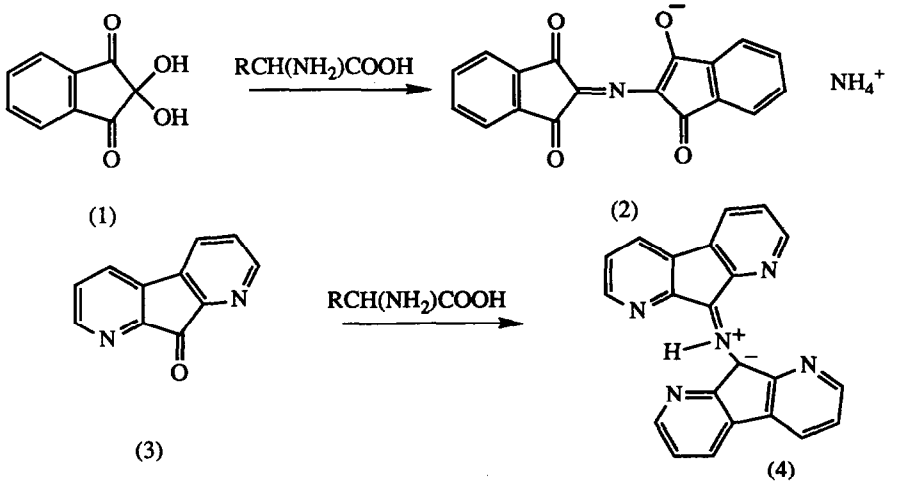
Moustafa F. Aly,[a] Galal M. El-Nagger,[b] Talaat I. El-Emary,[b] Ronald Grigg,[a,*] Saoud A.M. Metwally[c] and Sasikala Sivagnanam.[a]

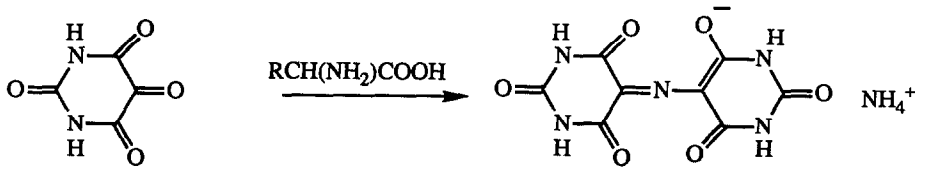
a. School of Chemistry, Leeds University, Leeds LS2 9JT
b. Department of Chemistry, Faculty of Science, Assiut University, Egypt.
c. Chemistry Department, United Arab Emirates University, United Arab Emirates.

Abstract. In situ formation of imines from α-amino acids and alloxan or pyrazolin-4,5-diones results in decarboxylation forming azomethine ylides which can be trapped as their cycloadducts with maleimides. In the absence of maleimides alloxan gives murexide and the pyrazoline-4,5-diones give rubazonic acid derivatives. The latter are reformulated as stable azomethine ylides. Methyl glycinate reacts with a pyrazolin-4,5-dione and maleimides to give cycloadducts via an ester stabilised azomethine ylide.

There are a number of reagents that react with α-amino acids to produce coloured products. Of particular note are ninhydrin (1)[2] and diazafluorenone (3)[3] both of which remove only the nitrogen atom from the α-amino acids in complex multistep mechanisms involving several types of azomethine ylides ultimately furnishing Ruhemann's purple (2) and the scarlet stable azomethine ylide (4) respectively.

The reagents (1) and (3) have been widely used to detect and quantitate α-amino acids[4] and/or to characterise latent fingerprints on paper or other suitable surfaces.[5,6] The similarity between the reactions of ninhydrin with α-amino acids and the previously discovered Strecker Degradation [the reaction of alloxan (5)



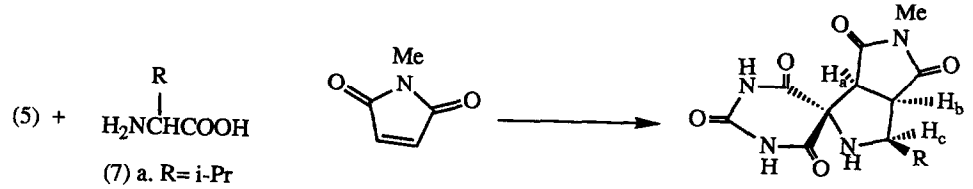


(5) (6)

with α-amino acids to give murexide (6)][7] was quickly noted by Ruhemann, the discoverer of ninhydrin. The detailed mechanism of the ninhydrin reaction has been the subject of numerous investigations[8] which culminated in our demonstration that several types of azomethine ylides are involved in the cascade process[2] leading to Ruhemann's Purple. We now report related studies on the Strecker Degradation.

In a seminal review Schonberg and Moubacher proposed the term 'Strecker Degradation' be applied to all reactions involving degradation of α-amino acids to aldehydes or ketones containing one carbon atom less no matter what the degrading agent used.[9] Since this all embracing definition encompasses a wide diversity of reagents and mechanisms this is both unfortunate and unrealistic in our view. In this paper we restrict the term 'Strecker Degradation'to those processes whose mechanisms closely pattern that of Strecker's original observation namely the reaction of alloxan with alanine to give acetaldehyde and carbon dioxide. This type of degradation is brought about by a wide variety of aldehydes, ketones and α-dicarbonyl compounds.

We observe that alloxan (5) reacts with valine (7a), phenylalanine (7b) and phenylglycine (7c) in aqueous ethanol in the presence of N-methylmaleimide (NMM) (8) to give cycloadducts (9a-c) in 20-42% yield. Changing the solvent to acetonitrile improved the yields of cycloadducts to 62-67%.

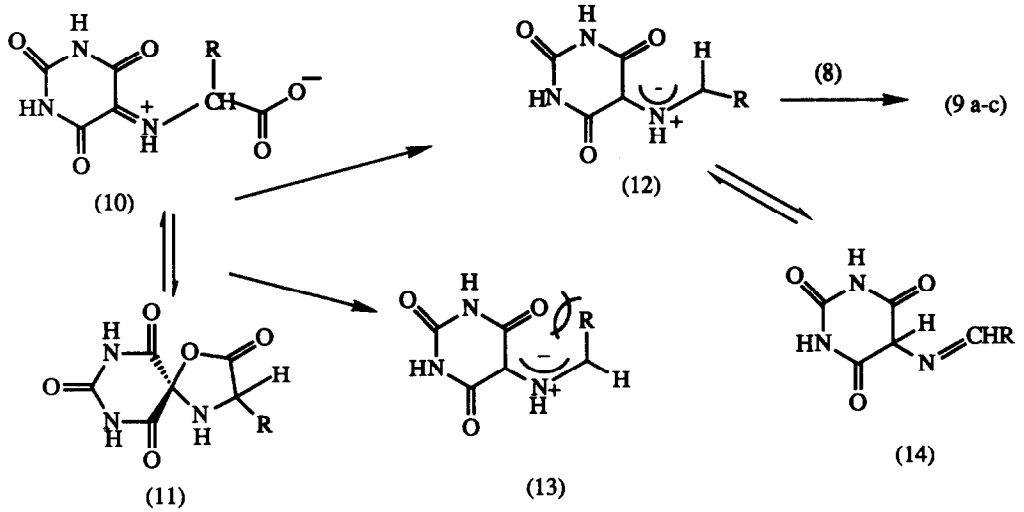


(7) a. R= i-Pr
b. R= CH_2Ph
c. R=Ph

(8)

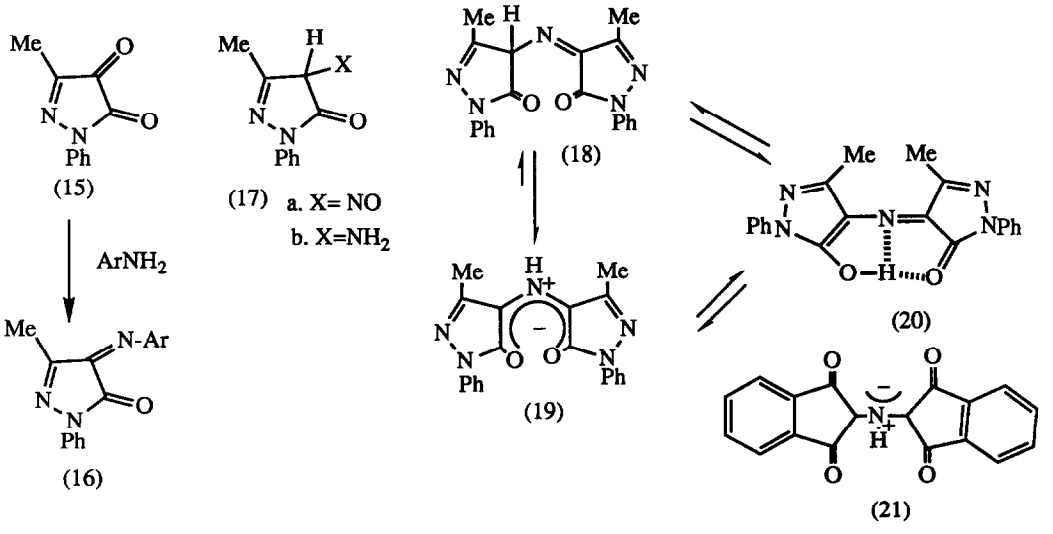
(9) a. R= i-Pr
b. R= CH_2Ph
c. R=Ph

The stereochemistry of the cycloadducts (9a-c) was established by n.O.e. difference spectroscopy. In particular, positive n.O.e.'s were observed on the signals for H_A and H_C when H_B was irradiated. The isolation of (9a-c) implicates an azomethine ylide intermediate in the Strecker Degradation. This intermediate can arise by direct decarboxylation of the zwitterionic imine (10) (Scheme 1) or via a concerted loss of carbon dioxide (1,3-dipolar cycloreversion) from the oxazolidin-5-one (11). Our previous studies on other carbonyl compound/amino acid combinations have provided evidence for the involvement of oxazolidin-5-ones analogous to (11) in azomethine ylide formation.[10] The stereochemistry of (9a-c) implicates azomethine ylide (12) (Scheme 1) and an endo-transition state for the cycloaddition rather than the alternative more strained azomethine ylide (13) which would require an exo-transition state to furnish (9). In the absence of a dipolarophile the azomethine ylide (12) undergoes 1,2-prototropy furnishing the imine (14) (Scheme 1) which releases the aldehyde (RCHO) on hydrolysis. Murexide (6) then arises from condensation of the liberated amine with alloxan. Thus the alloxan/α-amino acid cascade reaction mirrors that previously established for ninhydrin.[2]



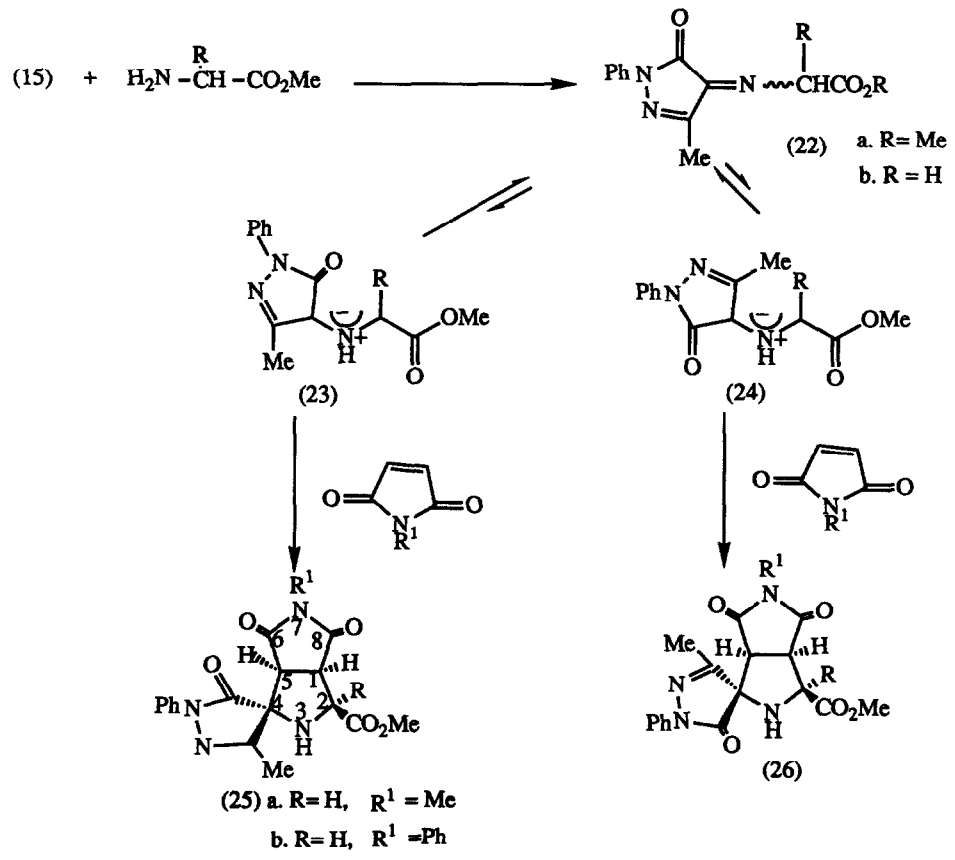
Scheme 1

1-Phenyl-3-methylpyrazoline-4,5-dione (15) reacts with primary aromatic amines to give the corresponding 4-imino derivatives (16) which have been widely used in colour photography.[11] Treatment of (17a) with Raney in the presence of air[12] or oxidation of (17b) with ferric chloride[13] lead to a red dye, rubazonic acid, which is also formed by condensation of (15) with (17b).[14] The red colour of rubazonic acid is clearly at odds with the proposed structure (18) and this parallels the situation for protonated Ruhemann's Purple which we showed by X-ray crystallography to be the stable azomethine ylide (21)[2]. Rubazonic acid would thus appear to be best formulated as the azomethine ylide (19), or a stereoisomer thereof, rather than an enolic isomer such as (20) although the potential for a solvent sensitive equilibrium between the two clearly exists.



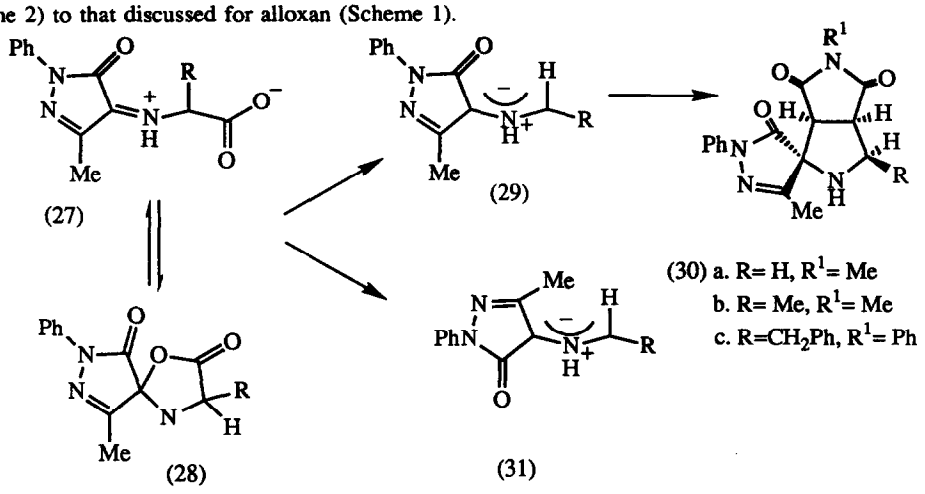
Two series of reactions were carried out with (15) in which it was reacted with both α-amino esters and α-amino acids in the presence of N-methylmaleimide (NMM) or N-phenylmaleimide (NPM).

Reaction of (15) with α-amino esters was best carried out in boiling toluene in the presence of di(n-butyl)tin(IV) dichloride. Under these conditions methyl glycinate reacted with both NMM and NPM to give a ca. 3:1 mixture of a single cycloadduct, (25a) and (25b) respectively, and rubazonic acid (19). Surprisingly rubazonic acid had not previously been prepared/detected in reactions of this type. The cycloadducts were isolated in ca. 50% yield.



The imine (22a), formed *in situ*, can give rise to four stereoisomeric azomethine ylides. Our extensive previous studies of azomethine ylides derived from primary α-amino esters indicates the ester group is always located cis to the azomethine ylide N-H due, it is believed, to hydrogen bonding between the NH and ester carbonyl group.[15] This observation reduces the potential configurational isomers of the azomethine ylide to (23) and (24). Maleimide dipolarophiles react with ester stabilised NH azomethine ylides stereospecifically to give endo-cycloadducts.[16] Thus the formation of a single endo-cycloadduct from (23) or (24) would lead to (25) or (26) respectively. The all cis arrangement of the pyrrolidine ring protons 1-H, 2-H and 5-H [see (23) for numbering] in the cycloadducts was established by n.O.e. studies, but the relative stereochemistry of the C(4)-spirocentre could not be established in this way. However, inspection of molecular models together with an X-ray crystal structure on a related cycloadduct (see below) indicates steric hindrance in (23) (CO/R interaction) is less than in (24) (N=CMe/R) and hence we favour structure (25) for the cycloadducts.[17]

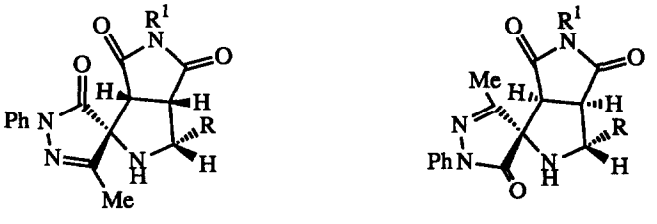
A second series of cycloadducts was prepared from the reaction of (15) with the α-amino acids glycine, alanine and phenylalanine in DMF at 105-110°C. Under these conditions the intermediate imine (22b) undergoes decarboxylation either via the zwitterion (27) or the oxazolidin-5-one (28) in an analogous sequence (Scheme 2) to that discussed for alloxan (Scheme 1).



(27) (29) (28) (31)

(30) a. R= H, R^1= Me
b. R= Me, R^1= Me
c. R=CH_2Ph, R^1= Ph

Scheme 2



(32) (33)

In each reaction a mixture of two stereoisomeric cycloadducts resulted in combined yields of 50-64%. The major isomer in each case is assigned structure (30a-c) based on n.O.e. data together with an X-ray crystal structure of (30c) (Figure 1). Since our previous work indicates endo-isomers predominate in such cycloadditions the major cycloadduct in each case must arise from azomethine ylide (29).

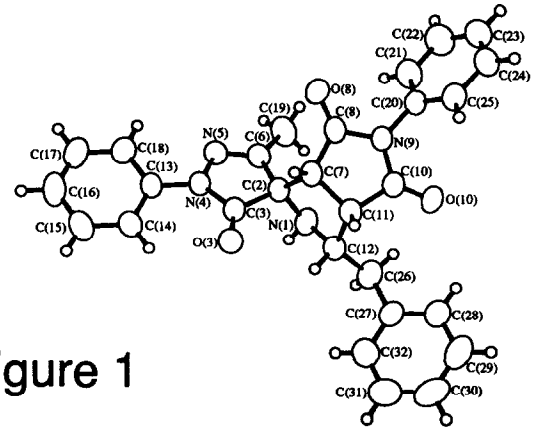
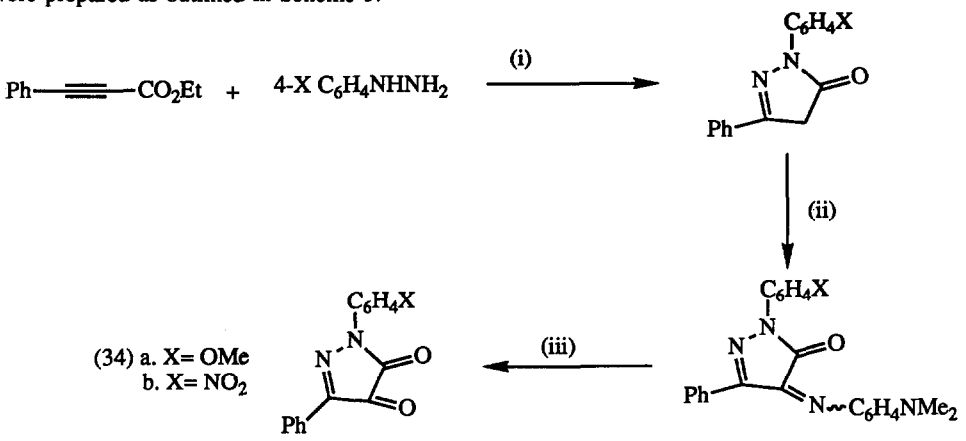


Figure 1

The minor cycloadducts (isomer ratios range from 2.4:1 to 6:1) could arise from endo cycloaddition of azomethine ylide (31) to NMM (NPM) or from exo-cycloaddition of NMM (NPM) to (29). The former would lead to (32) and the latter to (33). We have insufficient evidence to distinguish between these.

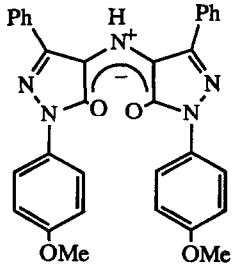
The reaction of α-amino acids with 1-phenyl-3-methylpyrazoline-4,5-dione (15) in boiling ethanol in the absence of a dipolarophile results in the crystallisation of rubazonic acid (19) as red needles from the reaction mixture. Evaluation of (15) as a reagent for the detection of latent fingerprints showed it was not as effective as ninhydrin.[18]

1-p-Methoxyphenyl-3-phenylpyrazoline-4,5-dione (34a) and 1-p-nitrophenyl-3-phenylpyrazoline-4,5-dione (34b) were prepared as outlined in Scheme 3.



Scheme 3. i) EtOH / 78°C / 2-3 dy. ii) Me_2N—⟨⟩—NO / EtOH / 78°C / 1 h

Pyrazoline dione (34a) reacted with alanine in boiling ethanol to give the corresponding rubazonic acid derivative (35) (or stereoisomer) (77%). In contrast (34b) failed to react with alanine under the same conditions. The reaction cascade leading from the pyrazoline diones and α-amino acids to the formation of rubazonic acid (19) and its derivative (35) is entirely analogous to that proposed by us for the formation of Ruhemann's Purple from ninhydrin.[2]



(35)

Experimental.

Melting points were determined on a Koffler hot-stage apparatus and are uncorrected. Infrared spectra were recorded on Perkin-Elmer Model 598 and 983 G instruments and refer to potassium bromide discs unless otherwise noted. Mass spectral data were obtained from a VG Autospec instrument operating at 70 eV.

Nuclear magnetic resonance spectra were recorded on QE300 and Bruker AM400 instruments operating at 300 and 400 MHz respectively. Unless specified deuteriochloroform was used as solvent. Microanalyses were obtained using a Carbo Erba MOD 11016 instrument. Preparative t.l.c. plates were prepared using silica gel 60 PF(Merck 7748). Column chromatography was performed with silica get 60(Merck 9385). Petroleum ether refers to the fraction with b.p. 40-60°C.

Reactions of α-Amino Acids with Alloxan.

General Procedure. A mixture of alloxan (3.12mmol), α-amino acid (3.12mmol) and NMM (3.73mmol) was boiled under reflux in acetonitrile (30ml) for 4-8h. The solution was filtered whilst hot to remove small amounts of murexide. On cooling the filtrate the cycloadduct crystallised out.

2-Isopropyl-4-(5′5′-spirobarbituryl)-7-methyl-6,8-dioxo-3,7-diazabicyclo[3.3.0]octane (9a). A reaction time of 4h using the general procedure with valine afforded the *product* (67%) as colourless plates, m.p. > 250°C (Found: C, 50.4; H, 5.2; N, 18.25. $C_{13}H_{16}N_4O_5$ requires C, 50.65; H, 5.25; N, 18.5%); δ ($CDCl_3$ + 3 drops TFA) 4.47 (dd, 1H, Hc), 4.12 (d, 1H, H_A), 3.9 (dd, 1H, H_B), 3.0 (s, 3H, NMe), 2.44 (m, 1H, C<u>H</u>Me_2), and 1.34 and 1.17 (2xd, 2x3H, CH<u>Me</u>$_2$); ^{1}H NOEDS (%): irradiation of the signal for H_B caused enhancement of the signals for H_A (7) and H_C (4); m/z (%) 308 (M^+,8), 265 (100), 197 (18), 152 (7) and 111 (32).

2-Benzyl-4-(5,′5′-spirobarbituryl)-7-methyl-6,8-dioxo-3,7-diazabicyclo[3.3.0]octane (9b). Prepared by the general procedure from phenylalanine with a reaction time of 6h. The *product* (61%) crystallised as colourless plates, m.p. > 250°C (Found: C, 57.65; H, 4.3; N, 16.0. $C_{17}H_{16}N_4O_5$ requires C, 57.3; H, 4.55; N, 15.7%); δ(DMSO-d_6) 11.5 (br s, 1H, NH), 7.25 (m, 5H, ArH), 4.4 (m, 1H, H_C), 3.6 (m, 1H, H_B), 3.4 (m, 1H, PhCH), 3.25 (d, 1H, H_A), 2.75 (s, 3H, NMe) and 2.6 (m, 1H, PhCH); ^{1}H NOEDS (%): irradiation of the signal for H_B caused enhancement of the signals for H_A (4) and H_C (6); m/z (%) 356 (M^+, 2), 313 (11), 265 (93), 215 (48), 151 (25), 128 (48) and 111 (60).

2-Phenyl-4-(5,′5′-spirobarbituryl)-7-methyl-6,8-dioxo-3,7-diazabicyclo[3.3.0]octane (9c). Prepared from phenylglycine by the general procedure with a reaction time of 8h. The *product* (66%) crystallised from aqueous DMF as colourless plates, m.p. > 250°C (Found: C, 52.95; H, 4.4; N, 15.75. $C_{16}H_{14}N_4O_5$ requires C, 53.3; H, 4.45; N, 15.55%); δ (DMSO-d_6) 11.4 (d, 2H, 2xNH), 7.38 (m, 5H, ArH), 5.6 (d, 1H, H_C), 3.7 (t, 2H, H_A and H_B) and 2.73 (s, 3H, NMe); ^{1}H NOEDS (%): irradiation of the signal for H_C caused enhancement of H_A + H_B (19) and of the ortho-phenyl protons (15); m/z (%) 342 (M^+, 3), 231 (65), 230 (13), 214 (64) and 111 (72).

2-Carboxymethyl-4-(4′,4′-spiro-1′-phenyl-3′-methyl-4′,5′-dihydro-5′-oxopyrazolinyl)-7-methyl-6,8-dioxo-3,7-diazobicyclo[3.3.0]octane(25a). A mixture of 1-phenyl-3-methylpyrazoline-4,5-dione(0.94g, 5mmol), N-methylmaleimide (0.66g, 6mmol), methyl glycinate hydrochloride (1.25g, 10mmol), triethylamine (1.01g, 10mmol) and di(n-butyl)tin(IV) dichloride (1.52g, 5mmol) in toluene (50ml) was boiled under reflux for 14h. The cooled reaction mixture was filtered and the filtrate evaporated under reduced pressure. The residue was extracted with ether (3x100ml) to separate the cycloadduct from the insoluble rubazonic acid. The combined ether extracts were washed with saturated aqueous sodium carbonate (200ml), water (200ml), dried (Na_2SO_4), and evaporated. Crystallisation of the residue from methanol afforded the *product* (0.93g, 50%) as colourless cubes, m.p. 235-237°C (Found: C, 58.05; H, 4.75; N, 15.05. $C_{18}H_{18}N_4O_5$ requires C, 58.35; H, 4.9; N, 15.15%); δ 7.83 (d, 2H, ArH), 7.4 (t, 2H, ArH), 7.21 (t, 1H, ArH), 5.05 (dd, 1H, J 6.75 and 5.15Hz, 2-H), 3.86 (s, 3H, OMe), 3.83 (t, 1H, 1-H), 3.55 (d, 1H, J 7.85Hz, 5-H), 3.03 (s, 3H, NMe), 2.6 (d, 1H, J 4.2Hz, NH) and 2.01 (s, 3H, Me); m/z (%) 370 (M^+, 54), 311 (24), 197 (100), 120 (22), 91 (21) and 77 (39); υ_{max} 3320, 1745, 1720, 1700 and
1600cm^{-1}.

2-Carboxymethyl-4-(4′,4′-spiro-1′-phenyl-3′-methyl-4′,5′-dihydro-5′-oxopyrazolinyl)-7-phenyl-6,8-dioxo-3,7-diazabicyclo[3.3.0]octane(25b). Prepared in an analogous manner to that described above using N-phenylmaleimide and methyl glycinate hydrochloride. The *product* (53%) crystallised from methanol as colourless needles, m.p. 210-212°C (Found: C, 63.6, H, 4.4; N, 12.75. $C_{23}H_{20}N_4O_5$ requires C, 63.9; H, 4.65; N, 12.95%); δ 7.86 (d, 2H, ArH), 7.53-7.20 (m, 8H, ArH), 5.16 (t, 1H, 2-H) 4.02 (t, 1H, 1-H), 3.86 (s, 3H, OMe), 3.73 (d, 1H, J 8.1Hz, 5-H), 2.68 (d, 1H, J 4.8Hz, NH), and 2.11 (s, 3H, Me); m/z (%) 432 (M^+,89), 259 (100), 173 (55), 119 (37), 91 (57) and 77 (83); υ_{max} 3335, 1745, 1725, 1670 and 1600cm^{-1}.

4-(4′,4′-Spiro-1′-phenyl-3′-methyl-4′,5′-dihydro-5′-oxopyrazolinyl)-7-methyl-6,8-dioxo-3,7-diazabicyclo[3.3.0]octane(30a). A solution of 1-phenyl-3-methylpyrazolin-4,5-dione (0.5g, 2.66mmol), glycine (0.2g, 2.66mmol), and N-methylmaleimide (0.354g, 3.2mmol) in DMF (20ml) was heated at 120°C for 17h. the solvent was then evaporated under reduced pressure to afford a residue which comprised a 4:1 mixture of two isomeric cycloadducts (^{1}H n.m.r.). The residue was triturated with dichloromethane and the insoluble material filtered off to afford the *major isomer* (30a) (0.43g, 52%) as a colourless solid, m.p. 253-255°C. The filtrate was evaporated to dryness and the residue purified by column chromatography (silica) eluting with dichloromethane and then ether to give the *minor isomer* (0.1g, 12%) which crystallised from dichloromethane-ether as colourless rods, m.p. 241-242°C.

30a. (Found: C, 61.35; H, 5.15; N, 17.95. $C_{16}H_{16}N_4O_3$ requires C, 61.55; H, 5.15; N, 17.95%); δ (C_5D_5N) 8.09 (dd, 2H, ArH), 7.42 (m, 2H, ArH), 7.21 (m, 1H, ArH), 4.39 (dd, 1H, J 8 and 9Hz, 2-H), 4.08 (d, 1H, J 8Hz, 5-H), 3.94 (t, 1H, J 8Hz, 1-H), 3.87 (d, 1H, J 9Hz, 2-H), and 3.09 and 2.21 (2xs, 2x3H, NMe and Me); ^{1}H NOEDS (%): irradiation of 5-H caused enhancement of the signal for 1-H(11) whilst irradiation of the signal for 1-H effected enhancements of the signals for the cis-2-H proton (δ 4.39) (7) and for 5-H (14); m/z (%) 312 (M^+,100), 284 (56), 255 (47), 201 (24) and 77 (78); υ_{max} 1700 and 1600cm^{-1}.

Minor isomer. (Found: C, 61.35; H, 5.05; N, 17.9); δ 7.86 (dd, 2H, ArH), 7.41 (dt, 2H, ArH), 7.21 (t, 1H, ArH), 4.09 (m, 1H, 2-H), 3.64-3.59 (m, 2H, 1-H and 2-H), 3.52 (d, 1H, 5-H), 3.07 (s, 3H, NMe) and 1.97 (s, 3H Me); m/z (%) 312 (M^+,100), 284 (47), 255 (41), 201 (12) and 77 (51).

2-Methyl-4-(4′,4′-spiro-1′-phenyl-3′-methyl-4′,5′-dihydro-5-oxopyrazolinyl-7-methyl-6,8-dioxo-3,7-diazobicyclo[3.3.0]octane(30b). Prepared from alanine and N-methylmaleimide in DMF at 110°C for 17h in a manner analogous to that described above. After evaporation of the DMF the residue was found to comprise a 2.4:1 mixture (^{1}H n.m.r.) of stereoisomeric cycloadducts. Column chromatography (silica) eluting with 1:1 v/v ether-petroleum ether afforded the major isomer (0.35g, 40%) which crystallised from ether-petroleum ether as colourless prisms, m.p. 138-139°C (Found: C, 62.35; H, 5.5; N, 16.95. $C_{17}H_{18}N_4O_3$ requires C, 62.55; H, 5.55; N, 17.15%); δ 7.85 and 7.4 (2xdd, 2x2H, ArH), 7.2 (t, 1H, ArH), 4.59 (m, 1H, 2H), 3.5-3.39 (m, 2H, 1-H and 5-H), 3.04 (s, 3H, NMe), 1.99 (s, 3H, Me) and 1.36 (d, 3H, J 6Hz, CHMe); ^{1}H NOEDS (%): irradiation of 2-H caused enhancement of the signals for 1-H and 5-H (11) and 2-Me (6); m/z (%) 326 (M^+, 19), 111 (25), 83 (70) and 57 (100); υ_{max} 1700 and 1600cm^{-1}.

2-Benzyl-4-(4′,4′-spiro-1′-phenyl-3′-methyl-4′5′-dihydro-5′-oxopyrazolinyl)-7-phenyl-6,8-dioxo-3,7-diazabicyclo[3.3.0]octane(30c). Prepared from phenylalanine and N-phenylmaleimide in DMF at 105°C for 14h in a manner analogous to that described above. After evaporation of the solvent under reduced pressure the residue, which comprised a 6:1 mixture of stereoisomeric cycloadducts (^{1}H n.m.r.) was purified by column chromatography (silica) eluting with 1:3 v/v ether-petroleum ether.

30c. The *major isomer* (0.7g, 30%) crystallised from methanol as colourless needles, m.p. 174-176°C (Found: C, 72.1; H, 5.1; N, 12.0. $C_{28}H_{24}N_4O_3$ requires C, 72.4; H, 5.2; N, 12.05%); δ 7.81 (d, 2H, ArH), 7.55-7.15 (m, 13H, ArH), 4.77 (m, 1H, 2-H), 3.67 (m, 2H, $PhCH_2$), 3.46 (dd, 1H, J 13.75 and 3.65Hz, 5-H), 2.7 (dd, 1H, J

13.75 and 10.25Hz, 1-H), 2.18 (s, 1H, NH) and 2.07 (s, 3H, Me); the X-ray crystal structure of this product is shown in figure 1; m/z (%) 464 (M^+,100) 373 (49), 291 (15), 173 (44), 119 (17), 91 (99) and 77 (58); υ_{max} 3340, 1720, 1700 and 1600cm^{-1}.

Minor isomer. Obtained as a colourless solid (0.22g, 9%), m.p. 97-99°C (Found: N, 12.15%); δ 7.74 (d, 2H, ArH), 7.48-7.06 (m, 13H, ArH), 4.59 (m, 1H, 2-H), 3.6 (d, 1H, J 9.9Hz, 5-H), 3.31 (m, 2H, $PhCH_2$), 2.79 (dd, 1H, J 13.35 and 9.5Hz, 1-H), 2.16 (s, 3H, Me) and 2.11 (s, 1H, NH); m/z (%) 464 (M^+,72), 373 (100), 291 (23), 173 (18), 119 (20), 91 (87) and 77 (69); υ_{max} 3340, 1720 and 1600cm^{-1}.

1-(4′-Methoxyphenyl)-3-phenyl-4,5-dihydro-4,5-dioxopyrazole(34a). (a). A mixture of 4-methoxyphenylhydrazine hydrochloride (1g, 5.73mmol) and triethylamine (0.58g, 5.74mmol) was boiled under reflux in ethanol (30ml) until all the solid dissolved (10-15min) at which point ethyl phenylpropiolate (1g, 5.74mmol) was added to the reaction. The resulting mixture was refluxed for 3 days and the precipitated 1-(4′-methoxyphenyl)-3-phenyl-4,5-dihydro-5-oxopyrazole (0.45g, 30%) was filtered off and washed with ethanol. Crystallisation from dichloromethane-ether afforded colourless needles, m.p. 200-201°C (Found: C, 72.3; H, 5.3; N, 10.5. $C_{16}H_{14}N_2O_2$ requires C, 72.15; H, 5.3; N, 10.5%); δ 7.86-7.83 (dd, 2H, J 7 and 2Hz, ArH), 7.77-7.74 (m, 2H, ArH), 7.47-7.44 (m, 3H, ArH), 6.97-6.94 (dd, 2H, J 7 and 2Hz, ArH) and 3.83 (s, 5H, $COCH_2$ and OMe); m/z (%) 266 (M^+,100), 251 (20), 223 (8), 121 (5) and 77 (19); υ_{max} 1665 and 1685cm^{-1}.

(b). A mixture of 1-(4′-methoxyphenyl)-4-imino-(4"-N,N-dimethylaminophenyl)-3-phenyl-4,5-dihydro-5-oxopyrazole (1g, 3.759mmol), *p*-nitrosodimethylaniline (6.564g, 3.759mmol) and 5% Na_2CO_3 (0.05ml), was boiled under reflux in ethanol (15ml) for 1h. The reaction mixture was then set aside in the fridge for 6h during which time the *product*, 1-(4′-methoxyphenyl)-4-imino-(4"-N,N-dimethylaminophenyl)-3-phenyl-4,5-dihydro-5-oxopyrazole, precipitated (0.96g, 63%). Crystallisation from dichloromethane-ether afforded dark red plates, m.p. 122-123°C (Found: C, 72.6; H, 5.6; N, 14.1. $C_{24}H_{22}N_4O_2$ requires C, 72.35; H, 5.55; N, 14.05%); δ 8.31-8.23 (m, 4H, ArH), 7.95 (dd, 2H, ArH), 7.49-7.43 (m, 3H, ArH), 6.98 and 6.74 (2xdd, 2x2H, ArH), 3.84 (s, 3H, OMe) and 3.16 (s, 6H, NMe_2); m/z (%) 398 (M^+,94), 235 (37), 135 (16), 121 (100) and 107 (20); υ_{max} 1670cm^{-1}.

(c). 1-(4′-Methoxyphenyl)-4-imino-(4"-N,N-dimethylaminophenyl)-3-phenyl-4,5-dihydro-5-oxopyrazole (1.0g, 2.51mmol) in ether (15ml) was vigorously stirred at room temperature for 1h with concentrated sulphuric acid (0.5ml) and water (5ml). The ether layer was separated, washed with water until neutral, dried ($MgSO_4$) and evaporated to dryness under reduced pressure. The residue was crystallised from dichloromethane-ether to afford the *product* 1-(4′-methoxyphenyl)-3-phenyl-4,5-dihydro-4,5-dioxopyrazole, (0.6g, 85%) as brown rods, m.p. 128-129°C. (Found: C, 68.6; H, 4.4; N, 10.2. $C_{16}H_{12}N_2O_3$ requires C, 68.55; H, 4.3; N, 10.0%); δ 8.16 (m, 2H, ArH), 7.86 (dd, 2H, ArH), 7.51 (m, 3H, ArH), 6.96 (dd, 2H, ArH) and 3.84 (s, 3H, OMe); m/z (%) 280 (M^+,5), 134 (12) and 121 (100).

1-(4′-Nitrophenyl)-3-phenyl-4,5-dihydro-4,5-dioxopyrazole (34b). (a). 4-Nitrophenylhydrazine and ethyl phenylpropiolate were boiled under reflux in ethanol for 48h to afford 1-(4′-nitrophenyl)-3-phenyl-4,5-dihydro-5-oxopyrazole (38%) which crystallised from dichloromethane-petroleum ether as yellow plates, m.p. 196-197° (Found: C, 63.75; H, 3.85; N, 15.05. $C_{15}H_{11}N_3O_3$ requires C, 64.05; H, 3.95; N, 14.95%); δ 8.33-8.24 (m, 4H, ArH), 7.81 (m, 2H, ArH), 7.51-7.26 (m, 3H, ArH) and 3.93 (s, 2H, CH_2CO); m/z (%) 281 (M^+,100), 145 (7), 136 (10), 103 (94) and 77 (41).

(b). Prepared from 1-(4′-nitrophenyl)-3-phenyl-4,5-dihydro-5-oxopyrazole, *p*-nitrosodimethylaniline and 8% aqueous sodium carbonate in boiling ethanol as described above. The *product* (57%) 1-(4′-nitrophenyl)-4-imino-(4"-N,N-dimethylaminophenyl)-3-phenyl-4,5-dihydro-5-oxopyrazole, crystallised from dichloromethane-ether as brown needles, m.p. 183-185°C (Found: C, 66.65; H, 4.65; N, 17.05. $C_{23}H_{19}N_5O_3$ requires C, 66.8;

H, 4.65; N, 16.95%); δ 8.37-8.24 (m, 8H, ArH), 7.48 (m, 3H, ArH), 6.75 (dd, 2H, ArH) and 3.2 (s, 6H, NMe_2); m/z (%) 413 (M^+,100), 383 (7) and 235 (73).

(c). A solution of the imine (above) in ether was hydrolysed by boiling with aqueous sulphuric acid as described for (34a). The *product* (52%) crystallised from dichloromethane-ether as brown plates, m.p. 177-178°C (Found: C, 60.85; H, 3.1; N, 14.3. $C_{15}H_9N_3O_4$ requires C, 61.0; H, 3.05; N, 14.25%); δ 8.37 (m, 2H, ArH), 8.23 (m, 4H, ArH) and 7.55 (m, 3H, ArH); m/z (%) 295 (M^+,35), 239 (100), 193 (10), 145 (15) and 136 (18); υ_{max} 1745 and 1595cm^{-1}.

Rubazonic Acid Analogue (35). A mixture of 1-(4′-methoxyphenyl)-3-phenyl-4,5-dihydro-4,5-dioxopyrazole (0.3g, 1.06mmol) and alanine (0.094g, 1.06mmol) in ethanol (10ml) was boiled under reflux for 1h. The *product* precipitated from the hot reaction mixture and on crystallisation from dichloromethane-petroleum ether afforded red needles (0.22g, 77%), m.p. 246-247°C (Found: C, 70.5; H, 4.7; N, 12.65. $C_{32}H_{25}N_5O_4$ requires C, 70.7; H, 4.65; N, 12.9%); δ 7.99-7.92 (m, 8H, ArH), 7.43-7.29 (m, 6H, ArH), 7.02 (d, 4H, ArH) and 3.87 (s, 6H, 2xOMe); m/z (%) 543 (M^+,18), 149 (6), 135 (23) and 121 (37).

Single crystal X-ray diffraction analysis of 30c - All crystallographic measurements were carried out at ambient temperature on a Stoe STADI4 diffractometer using graphite monochromated Copper K_α X-radiation (λ = 1.54184 Å). Two equivalent sets of data were collected in the range $4.0° < 2\theta < 130.0°$ using ω-θ scans. No significant variation was observed in the intensities of five standard reflections. Lorentz and polarisation corrections were applied to the data-set together with a semi-empirical absorption correction based on azimuthal ψ-scans.

The structure was solved by direct methods using SHELXS-86[19] and was refined by full-matrix least-squares (based on F^2) using SHELXL-93[20] which uses all data for refinement. The weighting scheme was $w = [\sigma^2(F_o^2) + (0.055P)^2 + 0.323P]^{-1}$ where $P=(F_o^2 + 2F_c^2)/3$. All non-hydrogen atoms were refined with anisotropic thermal parameters. All hydrogen atoms were constrained to predicted positions (C-H = 0.98, 0.97, 0.96 and 0.93Å for primary, secondary, methyl and aromatic hydrogens respectively). Restraints were applied to the phenyl rings such that they were flat with overall C_{2v} symmetry. Refinement included an isotropic extinction parameter x so that $F_c^* = k\,F_c[1 + 0.001 * x * F_c^2 * \lambda^3]^{-¼}$ where k is the overall scale factor. The residuals wR_2 and R_1, given below, are defined as $wR_2 = (\Sigma[w(F_o - F_c^2)^2] / \Sigma[wF_o^4])^{½}$ and $R_1 = \Sigma \, | \, |F_o| - |F_c| \, | \, / \, \Sigma \, |F_o|$. The latter is given for comparison with refinements based on F and uses reflections with $F_o > 4.0\,\sigma(F_o)$.

Crystal data - $C_{28}H_{24}N_4O_3$, 0.55 x 0.10 x 0.08 mm, M = 464.51, monoclinic, space group $P2_1/c$, a = 14.8305(9), b = 22.0220(12), c = 7.2480(3) Å, β = 92.312(4)°, U = 2365.3(2) Å^3, Z = 4, D_x = 1.304 Mg m^{-3}, μ = 0.700 mm^{-1}, $F(000)$ = 976.

Data collection - Scan speeds 1.5 - 8.0° min^{-1}, ω scan widths 1.05° + α-doublet splitting, $4.0 < 2\theta < 130.0°$, 7817 Data collected 3916 of which were unique, R_{int} = 0.0254, R_{sig} = 0.0343. There were 2551 reflections with $F_o > 4.0\,\sigma(F_o)$.

Structure refinement - Number of parameters = 321, isotropic extinction parameter x = 0.0024(2), goodness of fit s = 1.023, wR_2 = 0.1114, R_1 = 0.0395.

Non-hydrogen atomic co-ordinates are listed in Table 1. Selected bond lengths and angles are listed in Table 2. Supplementary data, which includes hydrogen co-ordinates and all thermal parameters together with complete bond lengths and angles, has been deposited at the Cambridge Crystallographic Data Centre and is available on request.

Table 1. Non-hydrogen atom co-ordinates (x 10^4) and equivalent isotropic thermal parameters ($Å^2$ x 10^3) for **30c** with estimated standard deviations (e.s.d.'s) in parentheses.

Atom	x	y	z	U_{eq}*
N(1)	6884.8(11)	1305.2(8)	4414(3)	53.6(10)
C(2)	6470.9(12)	1527.0(9)	2688(3)	46.2(11)
C(3)	6232.4(13)	2201.9(9)	2580(3)	45.9(11)
O(3)	6745.0(9)	2632.7(6)	2815(2)	58.4(8)
N(4)	5338.7(10)	2227.6(7)	2072(2)	46.8(9)
N(5)	4936.5(11)	1642.1(7)	1959(2)	50.9(9)
C(6)	5557.8(13)	1247.3(9)	2292(3)	50.1(11)
C(7)	7237.8(12)	1414.2(9)	1299(3)	46.9(11)
C(8)	7129.4(13)	836.9(9)	193(3)	48.4(11)
O(8)	6553.8(10)	733.2(7)	-988(2)	63.9(9)
N(9)	7816.2(10)	444.7(7)	726(2)	48.3(9)
C(10)	8381.0(13)	681.2(9)	2143(3)	53.3(11)
O(10)	9000.9(11)	401.3(8)	2859(2)	79.9(10)
C(11)	8106.2(13)	1328.0(9)	2516(3)	47.7(10)
C(12)	7841.0(13)	1480.2(9)	4499(3)	48.7(11)
C(13)	4774.1(13)	2738.6(9)	1726(3)	46.1(11)
C(14)	5047.7(14)	3312.2(9)	2292(3)	54.1(12)
C(15)	4482(2)	3799.4(11)	1946(4)	70(2)
C(16)	3654(2)	3726.4(12)	1077(4)	79(2)
C(17)	3386(2)	3152.9(12)	538(4)	73.5(14)
C(18)	3939.0(14)	2656.8(10)	842(3)	58.5(12)
C(19)	5334(2)	589.6(10)	2453(4)	70.3(14)
C(20)	7961.2(13)	-130.9(9)	-148(3)	47.6(11)
C(21)	7281.2(15)	-553.7(10)	-246(4)	65.6(13)
C(22)	7420(2)	-1093.4(11)	-1149(4)	83(2)
C(23)	8231(2)	-1216.4(12)	-1898(4)	83(2)
C(24)	8901(2)	-793.9(12)	-1800(4)	75.7(14)
C(25)	8770.0(14)	-243.0(10)	-936(3)	60.5(12)
C(26)	8330.4(14)	1166.1(11)	6132(3)	61.6(13)
C(27)	9229.8(14)	1441.1(10)	6696(3)	56.0(13)
C(28)	10025(2)	1147.3(12)	6411(3)	67.7(14)
C(29)	10847(2)	1412.5(15)	6951(4)	88(2)
C(30)	10864(2)	1968.4(15)	7788(4)	92(2)
C(31)	10075(2)	2265.6(14)	8098(4)	91(2)
C(32)	9262(2)	2004.3(12)	7561(3)	75(2)

*U_{eq} = 1/3 x trace of the orthogonalised U_{ij} matrix

Table 2. Selected bond lengths (Å) and angles (°) for **30c** with e.s.d.'s in parentheses

N(1)-C(2)	1.456(3)	N(1)-C(12)	1.468(3)
C(2)-C(6)	1.505(3)	C(2)-C(3)	1.529(3)
C(2)-C(7)	1.569(3)	C(3)-O(3)	1.223(2)
C(3)-N(4)	1.362(2)	N(4)-C(13)	1.419(2)
N(4)-N(5)	1.422(2)	N(5)-C(6)	1.283(3)
C(6)-C(19)	1.492(3)	C(7)-C(8)	1.508(3)
C(7)-C(11)	1.543(3)	C(8)-O(8)	1.206(2)
C(8)-N(9)	1.379(2)	N(9)-C(10)	1.399(3)
N(9)-C(20)	1.437(3)	C(10)-O(10)	1.207(2)
C(10)-C(11)	1.509(3)	C(11)-C(12)	1.542(3)
C(12)-C(26)	1.529(3)	C(13)-C(18)	1.383(3)
C(26)-C(27)	1.507(3)		
C(2)-N(1)-C(12)	108.7(2)	N(1)-C(2)-C(6)	111.8(2)
N(1)-C(2)-C(3)	117.3(2)	C(6)-C(2)-C(3)	100.6(2)
N(1)-C(2)-C(7)	101.9(2)	C(6)-C(2)-C(7)	118.9(2)
C(3)-C(2)-C(7)	107.1(2)	O(3)-C(3)-N(4)	126.7(2)
O(3)-C(3)-C(2)	127.3(2)	N(4)-C(3)-C(2)	106.0(2)
C(3)-N(4)-C(13)	129.9(2)	C(3)-N(4)-N(5)	112.3(2)
C(13)-N(4)-N(5)	117.8(2)	C(6)-N(5)-N(4)	107.9(2)
N(5)-C(6)-C(19)	120.9(2)	N(5)-C(6)-C(2)	113.1(2)
C(19)-C(6)-C(2)	125.7(2)	C(8)-C(7)-C(11)	105.6(2)
C(8)-C(7)-C(2)	114.2(2)	C(11)-C(7)-C(2)	105.2(2)
O(8)-C(8)-N(9)	125.0(2)	O(8)-C(8)-C(7)	126.5(2)
N(9)-C(8)-C(7)	108.5(2)	C(8)-N(9)-C(10)	112.7(2)
C(8)-N(9)-C(20)	123.4(2)	C(10)-N(9)-C(20)	123.8(2)
O(10)-C(10)-N(9)	123.3(2)	O(10)-C(10)-C(11)	127.7(2)
N(9)-C(10)-C(11)	109.0(2)	C(10)-C(11)-C(12)	116.9(2)
C(10)-C(11)-C(7)	103.8(2)	C(12)-C(11)-C(7)	105.5(2)
N(1)-C(12)-C(26)	110.0(2)	N(1)-C(12)-C(11)	100.7(2)
C(26)-C(12)-C(11)	119.5(2)	C(25)-C(20)-C(21)	120.6(2)
C(27)-C(26)-C(12)	114.6(2)		

We thank the Royal Society for a Developing Countries Fellowship (to M.F.A.) and the Home Office Forensic Science Research Laboratories for a studentship (to S.S.).

References.

1. Part 40. Grigg, R.; Rankovic, Z., and Somasunderam, A., *Tetrahedron*, 1993, in press.
2. Grigg, R.; Malone, J.F., Mongkolaussavaratana, T., and Thianpatanagul, S., *Tetrahedron*, 1989, **45**, 3849-3862.
3. Grigg, R.; Mongkolaussavaratana, T., Pounds, C.A., and Sivagnanam, S, *Tetrahedron Lett.*, 1990, **31**, 7215-7218.
4. Moore, S.; Stein, W.H., *J. Biol. Chem.*, 1948, **176**, 367-388; Schwett, R.S.; *ibid*, 1954, **208**, 603-613; Piez, K.A., Inevere, F., and Wolff, H.L., *ibid*, 1956, **223**, 687-697; Spackman, D.H.; Stein, W.H., and Moore, S., *Anal. Chem.*, 1958, 1190-1130; Dent, C.E.; *Biochem. J.*, 1948, **43**, 169-180.
5. Holsten, B.V.; Oden, S., *Nature*, 1954, **173**, 449-450; Oden, S.; Brit. Patent 767, 341 (1957); Almog, J.; *J. Foren. Sci.*, 1987, **32**, 1565-1573.
6. Pounds, C.A.; Grigg, R., and Mongkolaussavaratana, T., *J. Foren. Sci.*, 1990, **35**, 169-175.
7. Strecker, A.; *Annalen*, 1862, **123**, 353-365.
8. For reviews of the early work see: McCaldin, D.J.; *Chem. Rev.*, 1960, **60**, 39-51; Yuferov, V.P.; *Uspek. Biol. Khim.*, 1971, **12**, 62-71; Bolton, C.B.; Hanna, S.S., and Siehr, D.J., *Biochem. Educ.*, 1978, **6**, 4-5.
9. Schonberg, A.; Moubacher, R., *Chem. Revs.*; 1952, **50**, 261-277.
10. Aly, M.F., Grigg, R., Thianpatanagul, S., and Sridharan, V., *J. Chem. Soc., Perkin Trans. 1*, 1988, 949-955; Grigg, R.; Surendrakumar, S., Thianpatanagul S., and Vipond, D., *ibid*, 1988, 2693-2701; Grigg, R.; Idle, J., McMeekin, P., Surendrakumar, S., and Vipond, D., *ibid*, 1988, 2703-2713.
11. Brown, C.H.; Graham, B., Vittum, P.W., and Weissberger, A., *J. Am. Chem. Soc.*, 1951, **73**, 919-926.
12. Horyna, J.; Czech. Patent, 102347 (1962), through *Chem. Abs.*, 1963, **58**, 12573b.
13. Gysling, H.; Schwarzenbach, G., *Helv. Chim. Acta*, 1949, **32**, 1484-1504.
14. Knorr, V.L.; *Annalen*, 1887, **238**, 137-219; Prochazkova, L.; *Anal. Chem.*, 1964, **36**, 865-871.
15. For a review see: Grigg, R.; Advances in Cycloaddition, Edit. D.P. Curran, JAI Press, 1993, **3**, p. 161-204.
16. Amornraksa, K.; Grigg, R., Gunaratne, H.Q.N., Kemp, J., and Sridharan, V., *J. Chem. Soc., Perkin Trans. 1*, 1987, 2285-2296.
17. We are not suggesting there is an equilibrium between the azomethine ylides (23) and (24) but that the steric factors referred to come into play in the transition states leading from imine (22) to the azomethine ylides (23) and/or (24). Our previous studies[13] have shown that the highly reactive maleimides trap the azomethine ylides formed under kinetic control and do not permit equilibration.
18. We thank Mr. A. Pounds, Home Office Central Forensic Science Laboratory, Aldermaston, for this study.
19. Sheldrick, G.M.; *Acta. Crystallogr.*, 1990, **A46**, 467-473.
20. Sheldrick, G.M.; *J. Appl. Cryst.*, 1993, in preparation.

(*Received in UK* 17 *August* 1993; *accepted* 21 *October* 1993)