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X=Y-ZH COMPOUNDS AS POTENTIAL 1,3-DIPOLES. PART 23[1,2] MECHANISMS OF THE REACTIONS OF NINHYDRIN AND PHENALENE TRIONE WITH α-AMINO ACIDS. X-RAY CRYSTAL STRUCTURE OF PROTONATED RUHEMANN'S PURPLE, A STABLE AZOMETHINE YLIDE

Ronald Grigg[a], John F. Malone[a], Theeravat Mongkolaussavaratana[a] and Sunit Thianpatanagul[b]

(a. Chemistry Department, Queen's University, Belfast BT9 5AG, N. Ireland)
(b. Chemistry Department, Faculty of Science, Sikpakorn University, Nakon Pathom 7300, Thailand)

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Abstract. The ninhydrin reaction is shown to involve stereospecifically formed azomethine ylides of two types by trapping of the intermediates with maleimides as dipolarophiles. One type of azomethine ylide, in which the carboxyl group of the original α-amino acid is retained, is probably only important for glycine. The other type of azomethine ylide does not contain a carboxyl group and is formed from all ninhydrin positive α-amino acids via decarboxylative cycloreversion of an oxazolidin-5-one precursor. Phenalene-1,2,3-trione reacts with α-amino acids via a different mechanism despite the formal similarity of the two reagents. In this case decarboxylation occurs in a carbinolamine and azomethine ylides are not involved. An X-ray crystal structure of protonated Ruhemann's Purple shows it to be a stable N-H azomethine ylide, confirming the results of cycloaddition studies.

Ninhydrin (1) was first prepared by Ruhemann in 1910 and he subsequently discovered that it reacted with skin or with aqueous solutions of ammonia or primary amino acids to produce a deep blue or purple dye, Ruhemann's Purple (4)[3]. This colour reaction was subsequently developed first as a qualitative reagent for the detection of α-amino acids and proteins and subsequently as a sensitive reagent for the quantitative determination of amino acids.[4] Although ninhydrin is overwhelmingly used for α-amino acid estimation it is important to note that β-, γ-, δ- and even ε-amino acids can react under appropriate conditions.[5] Much later ninhydrin was utilised for the chemical development of latent fingerprints on paper.[6] It quickly assumed the role of the most important reagent for this latter application which depends on the production of Ruhemann's Purple by reaction of ninhydrin with α-amino acids in palmar secretions.

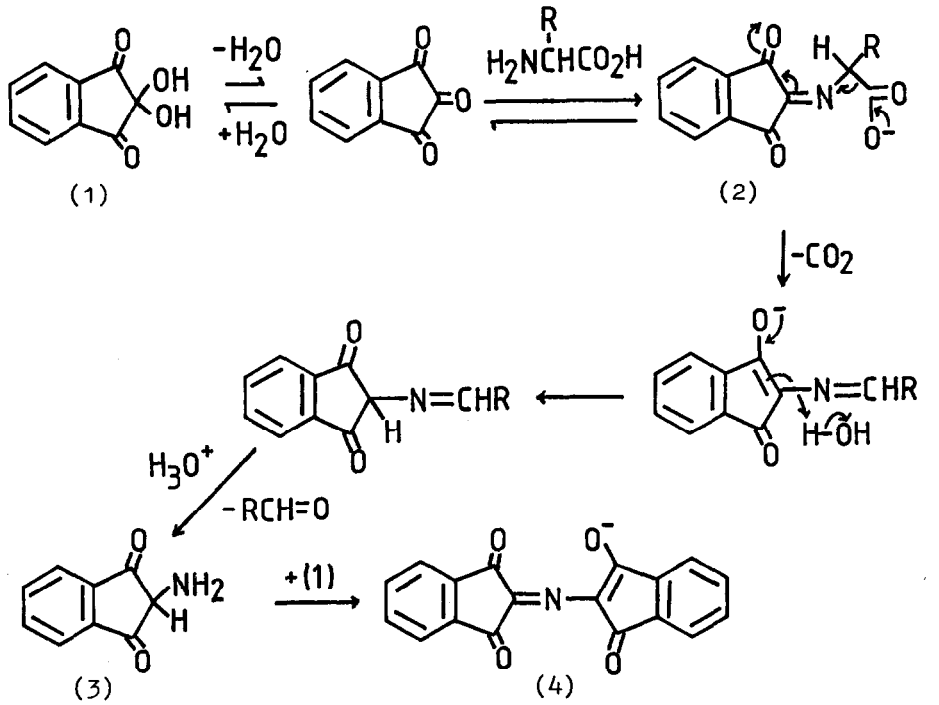
The surgical precision with which ninhydrin (1) removes only the nitrogen atom from the α-amino acid in forming (4), masks a complex multistep mechanism. Ruhemann quickly realised the similarity between his observations and the Strecker degradation.[7] Subsequently, among a plethora of studies, those of Grassman and Arnim[8], Abderhalden[9], and Moubasher et al[10], made important contributions to our understanding of the mechanism of the ninhydrin reaction. These and other studies of the ninhydrin reaction have been reviewed[11,12] and the most recent view of the mechanism is summarised in Scheme 1.[13] The formation of varying amounts of hydrindantin (5) in the ninhydrin reaction is suggested to involve side reactions of (3),[13] although the detection of free radical species in the reaction[12] prompts us to suggest a pinacol type coupling via (6) as a more plausible alternative. Indeed it is well known that (1) reacts with ascorbic acid to give (5)[14], presumably via (6).

Our interest in the ninhydrin reaction developed from our general studies of 1,2-prototropy in X=Y-ZH systems (7) ⇌ (8).[15] Thus we have shown that under appropriate conditions arylaldehydes and α-amino acids react to give azomethine ylides (9)-(11) (Scheme 2)† which retain the original α-amino acid carboxyl group[16] or which have lost the carboxyl group. The latter process proceeds via an oxazolidine-5-one (Scheme 2) and gives the anti-dipole (10), as opposed to the syn-dipole (11), stereoselectively or stereospecifically, depending on the structure of the α-amino acid and the aldehyde.[17-19] The studies summarised in Scheme 2 demonstrated the involvement of (9) in the racemisation of α-amino acids in the presence of aldehydes, provided a common mechanism for the Strecker degradation and decarboxylative transamination, and are strongly suggestive of the involvement of similar species in processes mediated by pyridoxal enzymes.[17,20] Ruhemann's original surmised relationship between the Strecker degradation and the ninhydrin reaction was reinforced by the later mechanistic studies summarised in Scheme 1, but the involvement of azomethine ylides in the ninhydrin reaction was unsuspected until our initial studies in 1984.[21]

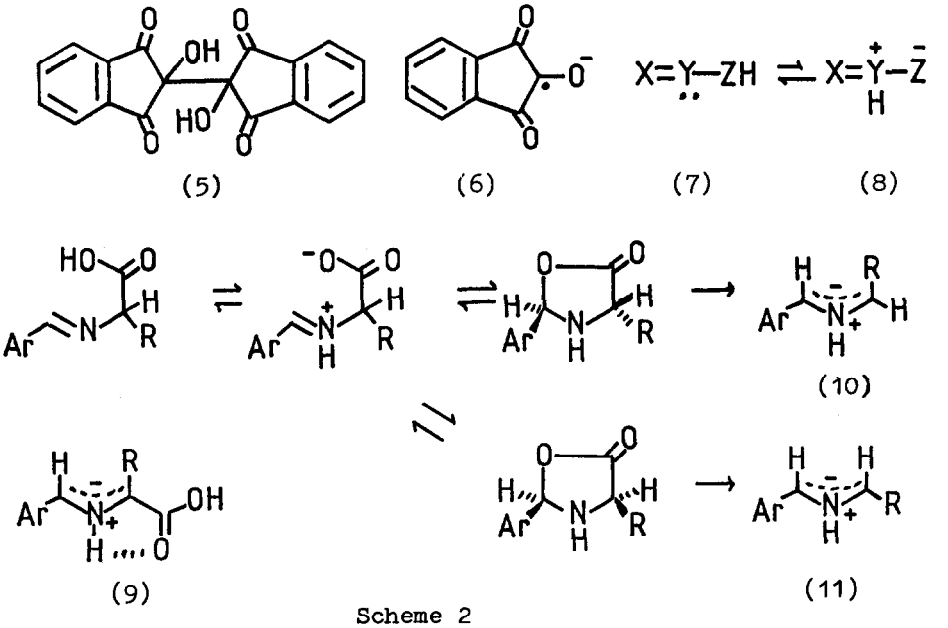
Our attention focussed initially on the imine (2) (Scheme 1) as a potential precursor of the two types of dipole (9) and (10)/(11) shown in Scheme 2. A range of α-amino acids (12a-g) were reacted with ninhydrin (1) and N-phenylmaleimide (NPM)(13a) or N-methylmaleimide (NMM)(13b). Reaction occurred under mild conditions, in methanol or aqueous methanol, to give the cycloadducts (14a-g) as single stereoisomers in good yield (Table).

The stereochemistry of representative examples of cycloadducts were

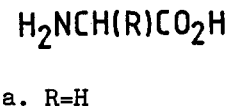
† Hydrogen bonding of the type depicted in (9) may involve a bridging water molecule.



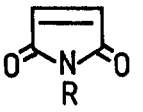
Scheme 1



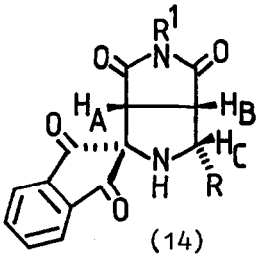
Scheme 2



(12) a. R=H
b. R=Me
c. R=CH_2Ph
d. R=3-indolylmethyl
e. R=$CHMe_2$
f. R=CH_2OH
g. R=Ph

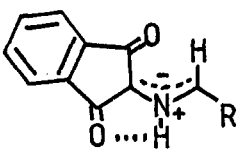


(13) a. R=Ph
b. R=Me



(14)

a. R=H, R^1=Ph
b. R=Me, R^1=Ph
c. R=CH_2Ph, R^1=Me
d. R=3-indolylmethyl, R^1=Ph
e. R=$CHMe_2$, R^1=Me
f. R=CH_2OH, R^1=Ph
g. R=R^1=Ph



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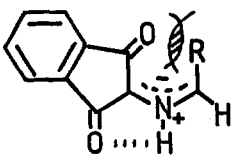

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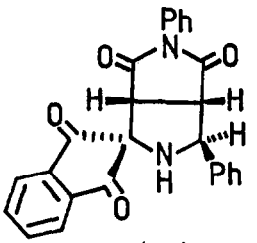


(17)

established using n.O.e. difference spectroscopy. Typical examples are provided by (14c) and (14e). Irradiation ($CDCl_3$) H_C of (14c) effects an 8% enhancement of the signal for H_B, whilst for (14e), irradiation of H_C effects a 15% enhancement of the signal for H_B. The cycloadducts (14a-g) could be derived from the cycloaddition of azomethine ylide (15) to the maleimide via an endo-transition state, or from azomethine ylide (16) via an exo-transition state. Our preference is for the former transition-state on the grounds that azomethine ylide (16) is expected to be energetically disfavoured by the steric clash between the R group and the syn-carbonyl group, and that this steric effect will manifest itself during the cycloreversion step generating the azomethine ylide. Thus loss of carbon dioxide from (18, partial structure) is accompanied by a rehybridisation of the sp^3 centres at C(2) and C(4) to sp^2 centres, whilst C(2) and C(4) participate in a disrotatory twisting motion.[18] Two disrotatory motions are possible, one of which (a) furnishes (15), whilst the other (b) would lead to (16). Steric control of electrocyclic ring opening processes is a well known phenomenon exemplified by the cyclobutene → butadiene system.[22]

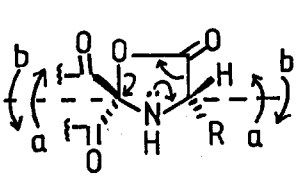
Table. Cycloadducts (14) from the reaction of (12a-g) with ninhydrin and (13a) or (13b)[a]

Amino acid	Dipolaro-phile	Temp. (°C)	Time (h)	Product	Yield[b] (%)
12a	13a	25	26	14a	81[c]
12b	13a	25	18	14b	83
12c	13b	64	2	14c	72
12d	13a	25	18	14d	70
12e	13b	64	2	14e	80
12f	13a	25	12	14f	48
12g	13a	64	2	14g	82[d]

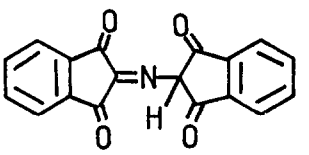
a. All reactions carried out in methanol or aqueous methanol using a 1:1:1 molar ratio of amino acid, ninhydrin and maleimide.
b. Isolated yield.
c. Product comprised a 1.6:1 mixture of (14a) and (14, R=CO_2H).
d. In addition ca. 8% of (17) was formed.

The formation of a 1.6:1 mixture of decarboxylated (14a) and undecarboxylated (14, R=CO_2H) cycloadducts in the reaction of glycine with ninhydrin and (13a) reflects the ability of 1,2-prototropy in (2, R=H; Scheme 1) to compete with oxazolidin-5-one formation and decarboxylative cycloreversion. The competition in this case presumably reflects an enhanced rate of prototropy due to the substantially lower pK_a of the methylene protons in (2, R=H) compared to the methine proton in (2, R=Me, CH_2Ph etc). Recent pK_a data for imines of α-amino acid esters lends support to this suggestion[23] and shows the glycine imine has a pK_a four units below that of the alanine imine. A similar 1:3.1 mixture of decarboxylated and undecarboxylated cycloadducts was obtained from the reaction of glycine with pyridoxal and NPM.[17] When ninhydrin and NPM are reacted with methyl glycinate in boiling acetonitrile the endo-cycloadduct (14, R=CO_2Me) is obtained in 74% yield.

We next attempted cycloaddition reactions of Ruhemann's Purple (4). Ruhemann's Purple is a stabilised aza-allylic anion usually with a sodium or ammonium counterion depending on the buffer used in the ninhydrin reaction. Kauffmann's extensive work with aza-allylic anions has shown that they participate in $4\pi + 2\pi$ anionic cycloaddition reactions.[24] However, attempts to prepare a cycloadduct from (4) and (13a) or (13b) were unsuccessful and encouraged us to investigate similar cycloaddition reactions



(18)



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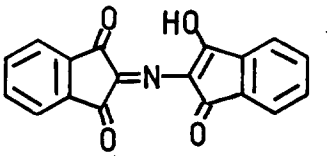

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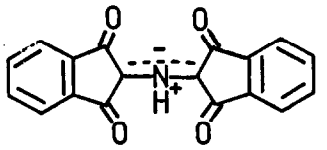

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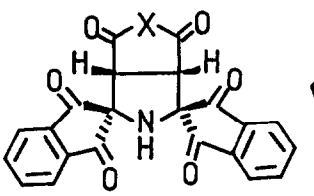
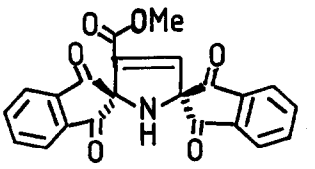




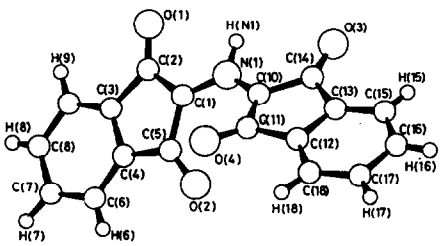
(23)

(22) a. X=NPh
b. X=O

with protonated Ruhemann's Purple. Protonation of (4), an ambident anion, could occur at carbon, oxygen or nitrogen to give (19), (20) or (21) respectively. A spectroscopic study (i.r., n.m.r., mass) of protonated Ruhemann's Purple concluded the protonated form had structure (19).[25] However, it seemed to us that the reported u.v. spectrum of protonated Ruhemann's Purple [$\lambda_{max}(CHCl_3)$ 485 (ε 16000)][25] was more in accord with structure (21), a structure considered but discarded by the previous workers.[25] Wigfield et al. commented that the ^{13}C n.m.r. spectrum of protonated Ruhemann's Purple consists of only five signals[25], showing equivalence about the central nitrogen atom. This observation could only be accommodated by structures (19) and (20) by assuming a rapid prototropic equilibration between equivalent structures, whereas it is readily accounted for by the azomethine structure (21). The reported chemical shift for the four equivalent carbonyl carbon atoms (181.7Hz) is very similar to that (183.4Hz) of the carbonyl carbon atoms 1 & 3 in the structurally unambiguous indantrione. Structure (21) is an azomethine ylide and we therefore attempted a cycloaddition reaction (DMF, 100°C, 20 min) between protonated Ruhemann's Purple and (13a). Work up afforded cycloadduct (22a)(89%). Analogous cycloadducts (22b) and (23) were obtained in good yield from maleic anhydride and methyl propiolate upon reaction in boiling dry THF or dry toluene. These latter two cycloaddition reactions failed to occur in hot DMF due to a faster competing hydrolysis of (21) to ninhydrin.

Thus the solution chemistry of protonated Ruhemann's Purple accords with its formulation as a stable N-protonated azomethine ylide (21). We therefore undertook an X-ray crystal structure determination of protonated Ruhemann's Purple and this unambiguously confirmed the N-protonated azomethine ylide structure (21) (Figure).

Crystal Data for (21): $C_{18}H_9NO_4$, M=303.3, monoclinic, a = 11.659(10) b = 19.398(16), c = 5.931(5)Å, β = 93.15(8)°, U = 1339.3Å^3, Z = 4, D_c = 1.504g cm^{-3}, F(000) = 624, space group $P2_1/n$ (No.14), Mo-K_α radiation, λ = 0.71069Å, μ(Mo-K_α) = 0.65 cm^{-1}. Diffraction data were recorded on a Stöe STAD1-2 diffractometer. The structure was determined and refined using the program SHELX.[26] All hydrogen atoms were located in the difference Fourier synthesis and were included in the refinement with isotropic vibration parameters. Non-hydrogen atoms were allowed to vibrate anisotropically. In the final stages the 998 reflections with $F > 6\sigma(F)$ were used to yield a final R value of 0.038.



Figure

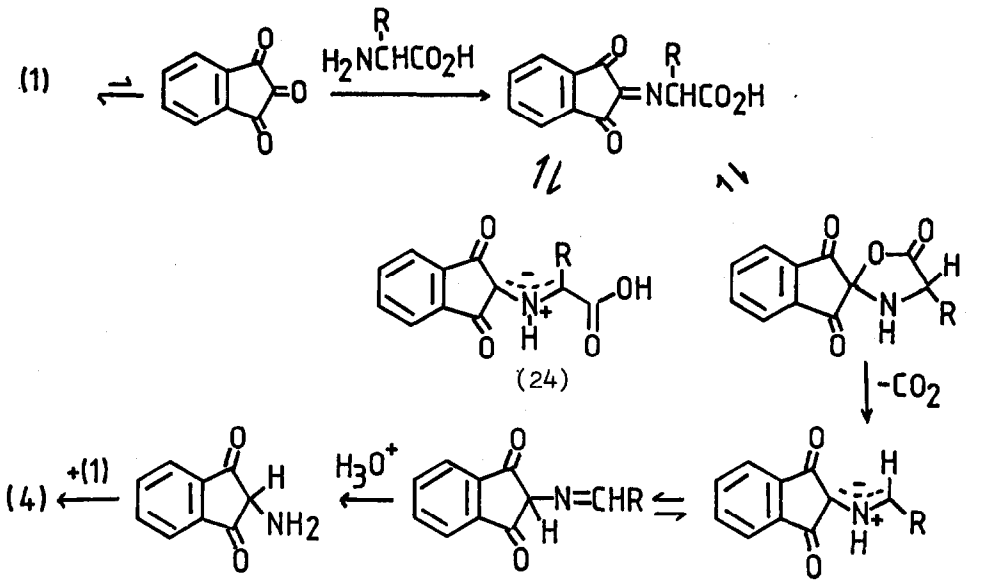
The X-ray results are completely consistent with structure (21), with no significant differences between the two halves of the molecule [e.g. N-C bonds are 1.340(5) and 1.346(4)Å]. The CNC bond angle is 127.9(3)° and repulsion between O(2) and O(4) is relieved by rotation of each indan-1,3-dione moiety by about 20° around the C-N bonds.

The work described above clearly demonstrates the intermediacy of two types of azomethine ylide in the ninhydrin reaction and leads to an expanded mechanistic scheme (Scheme 3) for the reaction which is an amalgam of Schemes 1 & 2.

The observation that the ninhydrin reaction occurs at an optimal pH of 4.5-5.2[27] supports the N-protonated azomethine ylide intermediates (24) and (25) rather than the corresponding aza-allylic anions.

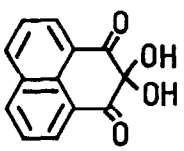
Phenalene-1,2,3-trione hydrate (26) is a ninhydrin like reagent that reacts with α-amino acids in a related decarboxylative way in that the α-amino acid is converted into the corresponding aldehyde with one carbon atom less. However in this case the reagent (26) is converted to (27a) rather than to the Ruhemann's Purple analogue (28).[28]

When (26) was heated with NMM and either glycine or alanine in aqueous



Scheme 3

methanol or aqueous acetonitrile no cycloadduct could be detected, but a precipitate was obtained. The spectral data of this product indicated it was the known amine (27b).[29] Failure to trap azomethine ylides (29) in these reactions suggests that although superficially similar, the reactions of ninhydrin, and of (26), with α-amino acids differ in important mechanistic detail. The simplest explanation is that dehydration of the initial phenalenetrione derived carbinolamine (30a) to the imine (31) is disfavoured compared to that of the analogous ninhydrin reaction intermediate. This is supported by a study of Dreiding models of (31) and the analogous ninhydrin imine which show much greater steric interaction between the carbonyl group and H- and R- groups of the amino acid moiety in the former case. The dehydration step is thus sterically retarded in the case of (30a) and this permits a faster direct decarboxylation of the carbinolamine (30a, arrows) to intervene leading directly to (27a), carbon dioxide, and imine, RCH=NH, which is rapidly hydrolysed to aldehyde and ammonia. The ammonia liberated in this latter step can then interact with (26) producing (32) which upon reaction with an α-amino acid via an analogous intermediate will produce (27b). The reaction of (26) and alanine or of (27a) and (27b) is reported[30] to give (33). However, the authors do not appear to have considered structure (34) which is a more mechanistically feasible product of the reaction of (27a) and (27b).



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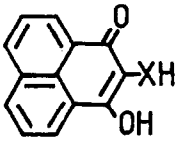
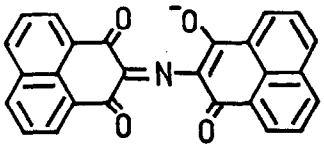


(27) a. X=O
b. X=NH



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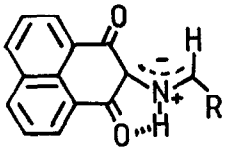

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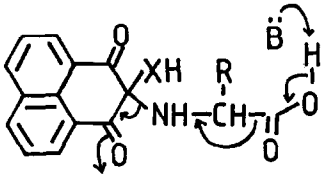
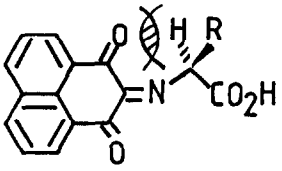


(30) a. X=O
b. X=NH
c. $X=\overset{+}{N}H_2$



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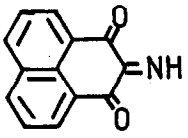

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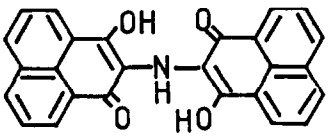

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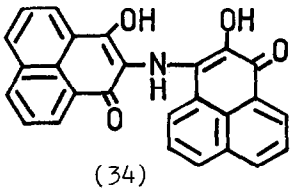

(34)

A number of kinetic studies[31] of the reaction of (26) with α-amino acids have failed to appreciate the important fundamental mechanistic difference between the phenalenetrione and ninhydrin reactions. Thus Strecker type reactions of α-amino acids can proceed via two different mechanisms. One of these, typified by ninhydrin and by pyridoxal, involves imine formation, followed by cyclisation to an oxazolidin-5-one and subsequent cycloreversion to an azomethine ylide and carbon dioxide. The other, typified by phenalene-1,2,3-trione, involves decarboxylation of an intermediate carbinolamine but does not involve azomethine ylide intermediates. The large proportion of (27b) formed in this latter process possibly reflects a lower energy charge assisted decarboxylation of the ammonium species (30c) or, more correctly, its corresponding zwitterion.

EXPERIMENTAL. General experimental details were as previously noted.[32]

Cycloadducts of Ninhydrin, α-Amino Acids and Maleimides

General Procedure. A mixture of ninhydrin (2mmol), N-methyl or N-phenyl-maleimide (2.02mmol) and α-amino acid (2.02mmol) in methanol or aqueous methanol (30-50ml) was boiled under reflux or stirred at room temperature for the time shown in the Table. The solvent was then evaporated to dryness and the residue crystallised from methanol, unless otherwise noted, to afford the pure cycloadducts. Yields are collected in the Table.

4-(2',2'-Spiro-1',3'-dioxoindanyl)-7-phenyl-6,8-dioxo-3,7-diazabicyclo[3.3.0]octane (14a) and 4-(2',2'-spiro-1',3'-dioxoindanyl)-7-phenyl-6,8-dioxo-3,7-diazabicyclo[3.3.0]octane-2-carboxylic acid (14, R=CO_2H). The reaction was carried out in 50% aqueous methanol and afforded a 1.6:1 mixture of (14a) and (14, R=CO_2H) which was separated by fractional crystallisation from methanol.

(14a) Colourless plates, m.p. 143-146°C (Found: C, 69.10; H, 3.90; N, 8.15 $C_{20}H_{14}N_2O_4$ requires C, 69.35; H, 4.05; N, 8.1%); δ ($CDCl_3$ + 1 drop TFA-d) 8.15-7.49 (m, 8H, ArH), 4.43 (m, 2H, H-2), 4.29 (m, 1H, 1-H) and 4.14 (d, 1H, J 9.1Hz, 5-H); m/z(%) 346 (M^+,100).

(14, R=CO_2H). Colourless prisms from chloroform, m.p. 240-242°C(d) (Found: C, 64.90; H, 3.90; N, 7.10. $C_{21}H_{14}N_2O_6$ requires C, 64.60; H, 3.60; N, 7.20%); δ ($CDCl_3$ + 1 drop TFA-d) 8.25-7.24 (m, 9H, ArH), 5.07 (d, 1H, J 7.4Hz, 2-H), 4.24 (t, 1H, 1-H) and 3.85 (d, 1H, J 7.9Hz, 5-H).

2-Methyl-4-(2',2'-spiro-1',3'-dioxoindanyl)-7-phenyl-6,8-dioxo-3,7-diazabicyclo[3.3.0]octane (14b). The reaction was carried out in 1:2 v/v aqueous methanol. A colourless solid (needles) which separated from the reaction mixture after 30min. proved to be the analytically pure cycloadduct, m.p. 233-234°C (Found: C, 70.00; H, 4.45; N, 7.85. $C_{21}H_{16}N_2O_4$ requires C, 70.00; H, 4.50; N, 7.75%); δ 8.05 and 7.68 (2xm, 9H, ArH), 4.26 (m, 1H, 2-H), 3.63 (m, 2H, 1-H and 5-H) and 1.54 (d, 3H, Me); m/z(%) 360 (M^+,100), 213(11), 198(45), 187(10) and 173(14).

2-Benzyl-4-(2',2'-spiro-1',3'-dioxoindanyl)-7-methyl-6,8-dioxo-3,7-diazabicyclo[3.3.0]octane (14c). The reaction was carried out in methanol. Pale yellow needles, m.p. 241-242°C (Found: C, 70.40; H, 4.90; N, 7.40. $C_{22}H_{18}N_2O_4$ requires C, 70.60; H, 4.85; N, 7.50%); δ 8.01 (m, 4H, ArH) 7.27 (m, 5H, ArH), 4.41 (m, 1H, 2-H), 3.49 (m, 3H, 1-H, 5-H and PhCH), 3.06 (s, 3H, NMe) and 2.85 (m, 1H, PhCH); m/z(%) 374 (M^+,19), 284(18), 283(100), 198(69) and 105(17); ν_{max} 3260 and 1685 cm^{-1}.

2-(3'-Indolylmethyl)-4-(2',2'-spiro-1',3'-dioxoindanyl)-7-phenyl-6,8-dioxo-3,7-diazabicyclo[3.3.0]octane (14d). The reaction was carried out in 1:2 v/v aqueous methanol. Yellow prisms, m.p. 197-198°C (Found: C, 70.90; H, 4.45; N, 8.40. $C_{29}H_{21}N_3O_4.H_2O$ requires C, 70.60; H, 4.70; N, 8.50%); δ8.03-7.10 (m, 14H, ArH), 4.62 (q, 1H, 2-H) 3.69 (t, 1H, 1-H), 3.60 (d, 1H, J 7.8Hz, 5-H), and 3.58 and 3.18 (2xdd, 2x1H, CH_2); m/z(%) 173(100) and 130(6).

2-Isopropyl-4-(2',2'-spiro-1',3'-dioxoindanyl)-7-methyl-6,8-dioxo-3,7-diazabicyclo[3.3.0]octane (14e). The reaction was carried out in methanol. Pale yellow needles, m.p. 184-185°C (Found: C, 66.00; H, 5.40; N, 8.40. $C_{18}H_{18}N_2O_4$ requires C, 66.25; H, 5.55; N, 8.60%); δ(C_6D_6) 7.56 (m, 2H, ArH), 6.89 (m, 2H, ArH), 3.65 (m, 1H, 2-H), 3.03 (d, 1H, J 7Hz,

5-H), 2.76 (t, 1H, 1-H), 2.70 (s, 3H, NMe), 2.03 (m, 1H, CHMe$_2$) and 1.20 and 0.93 (2xd, 2x3H, 2xMe); m/z(%) 326 (M^+,100), 284(17), 283(97), 198(87) and 105(16); ν_{max} 3320 and 1690 cm^{-1}.

2-Hydroxymethyl-4-(2',2'-spiro-1',3'-dioxoindanyl)-7-phenyl-6,8-dioxo-3,7-diazabicyclo[3.3.0]octane (14f). The reaction was carried out in 1:2 v/v aqueous methanol. Colourless prisms, m.p. 212-213°C(d) (Found: C, 65.90; H, 4.30; N, 7.30. $C_{21}H_{16}N_2O_5.0.5H_2O$ requires C, 65.45; H, 4.45; N, 7.27%); δ ($CDCl_3$ + 1 drop TFA) 8.08 and 7.39 (2xm, 9H, ArH), 4.98 (m, 1H, 2-H), 4.35 (m, 2H, CH_2O), 4.06 (t, 1H, 1-H) and 3.90 (d, 1H, J 8.8Hz, 5-H); m/z(%) 376 (M^+,53), 345(17), 198(100), 173(11) and 77(27).

2,7-Diphenyl-4-(2',2'-spiro-1',3'-dioxoindanyl)-6,8-dioxo-3,7-diazabicyclo[3.3.0]octane (14g) and (17). The reaction was carried out in 1:5 v/v aqueous methanol.

(14g). Pale yellow needles, m.p. 247-249°C(d) (Found: C, 73.95; H, 4.30; N, 6.65. $C_{26}H_{18}N_2O_4$ requires C, 74.20; H, 4.30; N, 6.55%); δ 8.12-7.26 (m, 14H, ArH), 5.53 (d, 1H, J 7.7Hz, 2-H), 3.65 (d, 1H, J 7.9Hz, 5-H) and 3.91 (t, 1H, 1-H); m/z(%) 422 (M^+,100), 275(23), 249(15) and 173(14); ν_{max} 3300, 1740 and 1705 cm^{-1}.

(17). Yellow rods from xylene, m.p. 230-233°C(d) (Found: C, 73.65; H, 4.60; N, 6.20%); δ 8.12-7.26 (m, 14H, ArH), 5.20 (d, 1H, J 7.3Hz, 2-H), 4.06 (d, 1H, J 10.1Hz, 5-H) and 3.82 (dd, 1H, 1-H); ν_{max} 3300, 1740 and 1705 cm^{-1}.

Methyl 4-(2',2'-spiro-1',3'-dioxoindanyl)-7-methyl-6,8-dioxo-3,7-diazabicyclo[3.3.0]octane (14, R=CO_2Me). A mixture of ninhydrin (356mg, 2mmol), NMM (222mg, 2mmol) and glycine methyl ester hydrochloride (251mg, 2mmol) in acetonitrile (15ml) was boiled under reflux for 12h during which time the product precipitated from the reaction mixture. The solid was collected by filtration and crystallised from DMF-ether to afford the product (520mg, 74%) as pale yellow needles, m.p. 261-262°C (Found: C, 59.50; H, 4.15; N, 7.95. $C_{17}H_{14}N_2O_6$ requires C, 59.65; H, 4.10; N, 8.20%); δ(DMSO-d_6) 8.04 (m, 4H, ArH), 4.70 (dd, 1H, 2-H), 3.85 (t, 1H, 1-H), 3.72 (s, 3H, OMe), 3.58 (d, 1H, J 7.5Hz, 5-H) and 2.81 (s, 3H, NMe); ^{1}H NOEDSY(%): irradiation of the signal for 2-H effected an enhancement in the signal for 1-H(18); m/z(%) 342 (M^+,94), 283(52), 198(100) and 197(33); ν_{max} 3280, 1725 and 1690 cm^{-1}.

Cycloadducts of Protonated Ruhemann's Purple.

2,4-Bis(2',2'-spiro-1',3'-dioxoindanyl)-7-phenyl-6,8-dioxo-3,7-diazabicyclo[3.3.0]octane (22a). A mixture of protonated Ruhemann's Purple (250mg, 0.8mmol)[25] and NPM (143mg, 0.8mmol) in DMF (15ml) was heated at 100°C for 20 min. On cooling the reaction mixture the cycloadduct (350mg, 88%) crystallised as yellow needles, m.p. 315°C(d) (Found: C, 70.20; H, 3.40;

N, 5.90. $C_{28}H_{16}N_2O_6$ requires C, 70.60; H, 3.40; N, 5.70%); δ (DMSO-d_6) 8.05 (m, 8H, ArH), 7.50 (m, 3H, ArH), 7.28 (m, 2H, ArH), 4.65 (s, 1H, NH) and 4.12 (s, 2H, 1-H and 5-H); m/z(%) 476 (M^+,100), 329(21), 273(17) and 105(25); ν_{max} 3350 and 1715 cm^{-1}.

2,4-Bis(2',2'-spiro-1',3'-dioxoindanyl)-6,8-dioxo-3-aza-7-oxabicyclo[3.3.0] octane (22b). A mixture of protonated Ruhemann's Purple (250mg, 0.8mmol) and maleic anhydride (97mg, 1mmol) in dry THF (40ml) was boiled under reflux for 12h. During this time the product (247mg, 75%) crystallised out as yellow needles, m.p. 255-256°C (Found: C, 65.60; H, 2.75; N, 3.25. $C_{22}H_{11}NO_7$ requires C, 65.85; H, 2.75; N, 3.50%); δ (DMSO-d_6) 8.04 (s, 8H, ArH), 4.79 (s, 1H, NH) and 4.31 (s, 2H, 1-H and 5-H); m/z(%) 401 (M^+,13), 329(8), 242(29), 170(61), 161(36) and 104(100); ν_{max} 3370, 1785 and 1715 cm^{-1}.

When the reaction was repeated in boiling dry toluene (40ml) for 1h the yield of product increased to 80%.

Methyl 2,5-bis(2',2'-spiro-1',3'-dioxoindanyl)-3-pyrroline-3-carboxylate (23).

A mixture of protonated Ruhemann's Purple (250mg, 0.8mmol) and methyl propiolate (76mg, 0.9mmol) in dry THF (40ml) was boiled under reflux for 12h. The solvent was then evaporated under reduced pressure and the residue crystallised from methylene chloride-hexane to afford the product (241mg, 75%) as yellow plates, m.p. 241-242°C (Found: C, 68.55; H, 3.15; N, 3.40. $C_{22}H_{13}NO_6$ requires C, 68.40; H, 3.15; N, 3.60%); δ 8.00 (m, 8H, ArH), 6.77 (s, 1H, =CH), 3.51 (s, 3H, OMe) and 3.21 (s, 1H, NH); m/z(%) 387 (M^+,93), 328(100), 300(26) and 272(19); ν_{max} 3340 and 1710 cm^{-1}.

Attempted Cycloaddition of Phenalene-1,2,3-trione Hydrate

A solution of glycine (164mg, 2.1mmol) in water (5ml) was added to a boiling solution of phenalene-1,2,3-trione hydrate (500mg, 2.1mmol) and NMM (256mg, 2.3mmol) in ethanol (30ml). The mixture was boiled under reflux for 10 min. during which time the 2-amino-3-hydroxyphenalen-1-one (27b)(285mg, 56%) precipitated from the solution as a light brown solid, m.p. 250°C (lit.[33] 260-265°C); δ (DMSO-d_6) 8.5 (m, 6H, ArH), m/z(%) 211 (M^+,100), 183(12) and 155(45); ν_{max} 3325 and 1635 cm^{-1}.

Evaporation of the reaction mother liquor and examination of the p.m.r. spectrum of ther residue showed no evidence for cycloadduct formation as judged by the absence of signals at δ 3-5. Similar results were obtained when the reaction was repeated in boiling acetonitrile. Use of alanine in place of glycine also gave the same result.

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