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N-Quaternary Compounds

Part IX. Pyridinium-3-oxide Derivatives from Amino Acids

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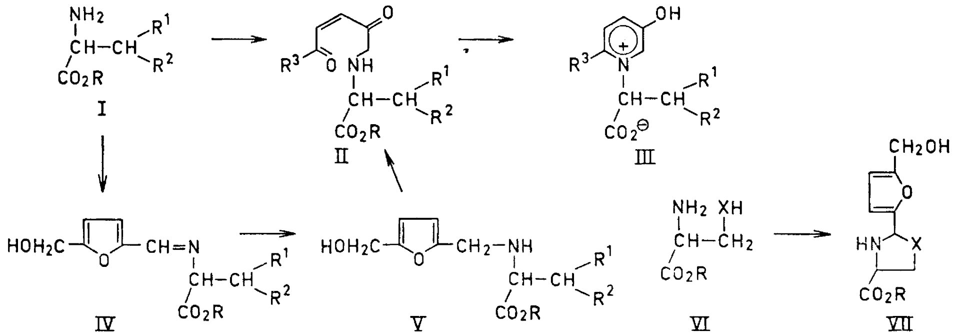
It has been shown that *N*-(5-hydroxymethylfurfuryl)amino acid esters can be synthesized by catalytic hydrogenation of the condensation product between amino acid esters and 5-hydroxymethylfurfural, by a Mannich type reaction between furfuryl alcohol and an amino acid, and by hydroxymethylation of a furfurylamino acid. Acid catalyzed rearrangement leads to 6-methylpyridinium-3-oxide derivatives of the corresponding amino acids. Such derivatives of simple amino acids are optically active. The cysteine analogue was best synthesized using the *S*-benzyl protecting group which then was removed by HBr in acetic acid. The hydroxyl group in serine was lost by a β-elimination process with the formation of the acrylic acid derivative. The pyridinium oxides showed strong UV absorption at 300 and 330 mμ in acidic and alkaline solution, respectively. NMR data are discussed and used in conformational assignments.

Condensation between 3-hydroxypyridine or 6-methyl-3-hydroxypyridine and α-halogeno or α-tosyloxy carboxylic acid derivates leads to *N*-quaternisation.[1] Larger *ortho* substituents in the pyridine such as a bromine atom or a thioether group leads to *O*-alkylation.[2] Studies of the stereochemistry of the condensation product in the Menschutkin reaction using optically active α-substituted acid derivatives require access to the same derivatives with known configuration. We herein report the synthesis of such derivatives from amino acids of known configuration in such a way that the configuration at the asymmetric carbon is not affected. Through the visualized intermediate (II) the amino acid nitrogen becomes the annular nitrogen while the carbons of the pyridine ring are derived from a very reactive *cis*-1,4-diketo-alkene as present in II. *cis*-1,4-Diketo-alkenes are best obtained *in situ* by acid catalysed ring opening of furans at the right oxidation level and Clauson-Kaas *et al.*[3] have shown that furfurylamines substituted in the furan ring will rearrange to 3-pyridinols. The desired oxidation level can be reached either by oxidation of the α-carbon in a side-chain in the 5-position in a furfurylamine, or by oxidation of the furan ring itself. The latter alternative leads to a general method for the synthesis of pyridinium-3-oxides carrying any desired substituent in the 6-position and is not restricted to an alkyl or aralkyl group as in the case under consideration. Thus the desired oxidation level was obtained by using the 5-hydroxymethyl derivative of *N*-substituted furfurylamines (V). The latter were synthesized from amino acid esters *via* Schiff base formation with 5-hydroxymethylfurfural followed by hydrogenation. The hydrogenations were done catalytically in methanolic solution at atmospheric pressure and room temperature over Raney-Ni or 5 % palladium on charcoal.

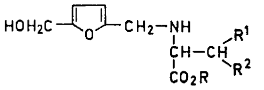


Invariably some reduction of the aldehyde group occurred especially if the uptake of hydrogen by the ylidine was slow. Excess of furfural was therefore often used. The optimum pH of the solution was 8—9 for esters of the simple amino acids alanine, valine, and phenylalanine. The pH fell to about 6 during the reaction. The optimum pH for the ester of S-benzyl cysteine was about 7. The use of the amino acid itself in this reaction led to poorer yields and more heterogeneous products.

The optical rotations of the phenylalanine derivative (Vc) prepared at various pH values are given in Table 1. The optical purity is highest at pH 9. At higher pH values base catalysed racemisation occurs. This is best illustrated for the alanine derivative (Va) prepared at pH 12—13 in which case the rotation was only about 20 % of that of the product prepared at pH 9. These derivatives are also optically unstable at elevated temperatures. Thus on distillation at reduced pressure the rotation of Vc was reduced to about a third of the original value.

Branching on the β-carbon in the amino acid, the example chosen being valine, resulted in a reduced rate of hydrogen uptake and formation of larger amounts of 2,5-dihydroxymethylfuran. This is because of the steric hindrance due to both the alkyl groups and the carboxylate group. Steric hindrance is also taken to account for the failure of O-*tert*-butylserine *tert*-butyl ester to undergo condensation while the corresponding cysteine derivative furnished the expected furfurylamine (Ve) in good yield. Therefore the difference in the size of the sulphur and oxygen atoms, the covalent single-bond radii begin 1.04 and 0.66 Å respectively,[4] must be sufficient to remove the bulky *tert*-butyl group far enough for unshielding the amino group.

Table 1. Optical rotations measured at 25°.

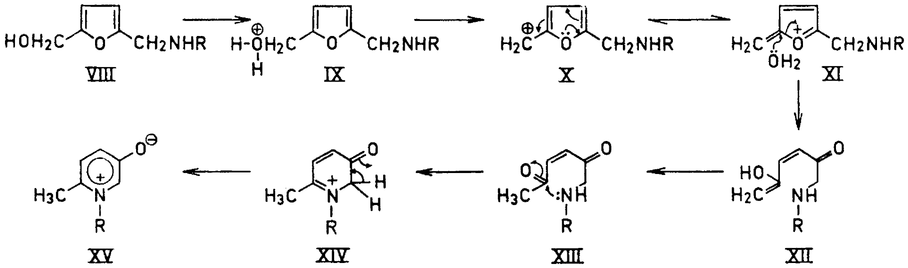


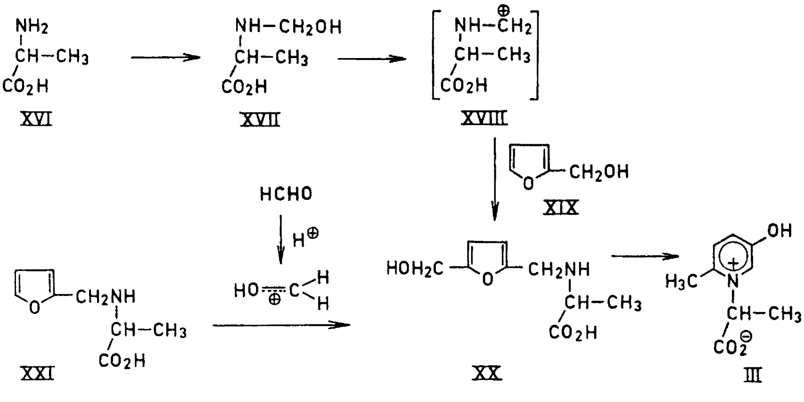
Comp.	Substituents			Configu- ration	Initial pH of the reaction	Optical rotation in MeOH	
V	R	R^1	R^2			$[\alpha]_{589}$	$[\alpha]_{546}$
a	CH_3	H	H	L	9	−31.3	−36.8
a					12−13	− 6.0	− 7.7
b	CH_3	CH_3	CH_3	L	9	− 8.6	−12.7
c	CH_3	H	C_6H_5	L	5	−13.6	−15.0
c					9	−21.0	−25.5
c					11	−17.6	−22.1
c					9 (distilled)	− 6.9	− 8.4
d	CH_3	H	$-SCH_2-C_6H_5$	L	7	− 2.8	− 4.0
e	$C(CH_3)_3$	H	$-S-C(CH_3)_3$	L	8.5	−10.9	−13.7
f	CH_3	H	OH	L	8.5	− 3.0	− 4.5
f				D	8.3	+ 6.3	+ 8.9
g	CH_3	H	OAc	D	8.2	+ 8.9	+12.9

A functional group on the β-carbon of the amino acid would be expected to lead to complications unless protected. Thus the reaction in the case of serine was difficult to effect despite the use of large excess aldehyde, the product being heavily contaminated by the amino acid. To exclude the possibility of oxazolidine ring formation (VII, X=O) the serine was *O*-acetylated since the *O*-acetyl group is stable towards hydrogenolysis.[5] *O*-Acetyl serine methyl ester was prepared by saturation of a solution of serine methyl ester in acetic anhydride and acetic acid with gaseous hydrogen chloride. An attempted esterification of *O*-acetyl serine [8] in methanolic hydrogen chloride resulted in deacetylation. The pH of the solution during furfurylamine formation was around 8 to minimize O→N acetyl migration. However, the hydrogen uptake was unsatisfactory and the reaction product was partly deacetylated. The more stable *O*-*tert*-butyl protecting group was therefore introduced. *O*-*tert*-Butylserine *tert*-butyl ester was synthesized by reacting isobutylene with serine hydrosulphate in dioxane,[8] but this bulky molecule could not be made to condense with the furfural.

In the case of cysteine derivatives 5 % palladium on charcoal or deactivated Raney-Ni, W-5,[9] could be used without desulphurisation, the former catalyst being the better. With cysteine methyl ester itself no hydrogen uptake occurred, due in part to poisoning of the catalyst but mainly to the ease with which the thiazolidine ring (VII, X=S) is formed.[10] Conversion of cysteine into a disulphide appeared to be a possible method for S-protection provided the hydrogenation proceeded faster than the hydrogenolysis of the disulphide bond. Some of the wanted product was undoubtedly formed from cystine methyl ester, but the reaction was too slow to be preparatively useful, probably because the catalyst was too heavily poisoned. *S*-Tetrahydropyranyl cysteine methyl ester was then prepared,[11] but the pyranyl group was partially lost under the conditions of the experiment. On the other hand, both *S*-benzyl methyl ester and *S*-*tert*-butyl *tert*-butyl ester of cysteine[12] gave good yields of the corresponding furfurylamines (Vd, Ve).

The conditions for the rearrangement of the furfurylamines (V) to the pyridinium 3-oxides (III) were studied using the phenylalanine derivative. Qualitatively the reactions were followed by chromatography and quantitatively by measuring the characteristic long wave UV absorption band of the pyridinium-3-oxide ring at about 330 mμ in NaOH and about 300 mμ in HCl. At 100° in 4 N HCl the reaction was over in 4 h, in N HCl in 7 h, and in 0.2 N HCl in 25 h, the time becoming progressively longer with weaker acid strength. The rearrangement was even found to proceed slowly in distilled water. Preparatively the reactions were run in N HCl as this seemed to give a fairly clean product coupled with convenient reaction time. The above findings indicate that the reaction is initiated by protonation of the more basic hydroxyl oxygen followed by loss of water and opening of the ring as in the case of furfuryl alcohol[13,14] and not protonation of the much less basic ether oxygen or its α-carbon.[15] The postulated mechanism for the reaction is shown below:





This mechanism requires the presence of water as the nucleophile to open the ring (XI) since the amino acid nitrogen at this stage is physically prevented from attacking at the electrophilic site. However, once the ring is open the nitrogen can freely move and attack the carbonyl carbon (XIII). This mechanism would appear to be supported by our failure to effect the reaction in polyphosphoric acid which is a very strong water absorbent.

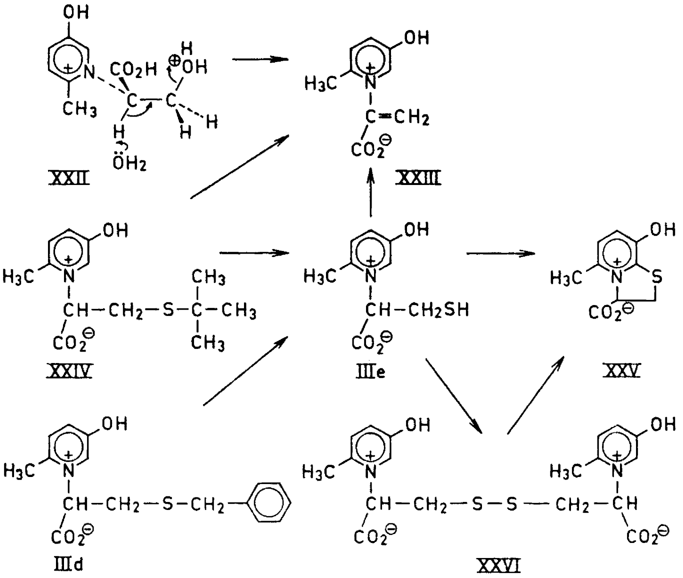
The N-furfuryl amino acids (V) should also be available by a Mannich type reaction between the furan XIX, formaldehyde, and an amino acid. In this way the catalytic hydrogenation would be avoided. Secondary amines are normally used in the Mannich reaction to prevent dialkylation but with the large groups present in the furfurylamine (V) first formed, any further substitution should be unfavourable. The reaction was attempted using alanine and varying the acid strength. Formation of the carbonium ion (XVIII) is acid catalysed, but on the other hand a high acid concentration will decrease the nucleophilicity of the α-carbon in the furan ring due to protonation and thereby reduce the reactivity. It was found that the acid strength necessary to effect the reaction was sufficient to catalyse the rearrangement of V so that the pyridinium oxide (III) was obtained directly, albeit in poor yield.

The hydroxymethyl function can also be introduced into the furan ring (XXI [16]→XX) by acid catalysed hydroxymethylation with formaldehyde as was very recently reported [17] in the case of 2-furfurylamine itself. Again the acid strength necessary for catalysing the hydroxymethylation caused the primary product (V) to react further to the pyridinium-3-oxide (IIIa).

The yields in the acid rearrangements varied between 85 and 40 % as measured by UV absorption of the reaction mixture. After isolation from the tarry product mixture, using such techniques as crystallisation of the hydrochloride, phenol extraction, and anion exchange chromatography, the yield was less than one half of the above values.

Protecting groups sensitive to acids are removed during the acid rearrangement. The *O*-acetyl group in the serine derivative (Vg) was thus lost. However, the initially formed serine pyridinium salt (IIIf) was found to have suffered β-elimination of the hydroxyl group, yielding the corresponding acrylic acid (XXIII). This was shown by the loss of the normal ABX system of the amino acid side-chain in the NMR spectrum in deuterium oxide and the presence in the olefinic region of two doublets at 3.35 and 3.90 τ ($J=1.5$ cps). Water elimination was further supported by complete loss of optical activity. The methine proton in XXII is highly activated by the neighbouring quaternary nitrogen, the carboxyl group and the carbon carrying the hydroxonium intermediate and is therefore readily abstracted by water molecules which results in β-elimination.

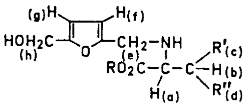
In the cysteine series the *S*-*tert*-butyl group is acid labile, the product formed in the rearrangement being the thiol (IIIe) and the acrylic acid (XXIII). The latter could arise both by β-elimination of *tert* butylthiol or *via* the normal rupture of the sulphur-*tert*-butyl bond followed by elimination of H_2S. The latter path-way would be supported by the behaviour of the *S*-benzyl derivative as discussed below besides the finding that in a separate experiment the acrylic acid (XXIII) was formed from IIIe when this molecule was heated



in dilute acid. The total yield of pyridinium-3-oxides in this reaction was about 40 % as measured by UV absorption, but the working-up of the reaction mixture was difficult, so this approach was dropped in favour of the reaction with the *S*-benzyl group. The latter was found to be the best S-protecting group. It did not suffer hydrogenolysis and was stable under the acid rearrangement simplifying the purification of the final product. The benzyl group was simply and cleanly removed by allowing a solution in acetic acid saturated with HBr to stand for 2 days at room temperature. Various attempts to prepare the dihydrothiazolo[3,2-a]pyridinium-8-oxide derivative (XXV) by oxidative cyclisation of the thiol (IIIe) in acidic or alkaline solution met with little success. Nor could the disulphide (XXVI), prepared by bubbling air through a weakly alkaline solution of the thiol (IIIe), be made to cyclise by acid breakage of the disulphide linkage.

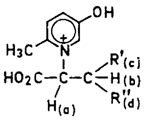
The NMR data of the *N*-furfuryl amino acids are given in Table 2. The methylene protons next to the asymmetric carbon in the amino acid moiety resonate as a doublet while the coupled methine proton resonate as a triplet instead of an octet and quartet, respectively, as in an ABX system, Therefore the magnetically non-equivalent methylene protons must have approximately the same chemical shifts. This was also true for the parent amino acid derivatives with the exception of S-*tert*-butyl cysteine *tert*-butyl ester where the full ABX system was apparent, the chemical shifts being 7.15, 7.20 and 6.47 τ. The differences in the chemical shifts of the methylene protons indicate that a conformation is very much preferred, presumably that in which the large *tert*-butyl groups are placed *anti* to each other. In the pyridinium-3-oxides (Table 3) which have large groups on the β-carbon such as IIIc and IIId, the methylene protons have different chemical shifts. With large groups

Table 2. NMR spectra in $CDCl_3$.



Comp.	Substituent			Chemical shift in τ values									Coupling constants in cps					
V	R	R′	R″	$H_{(h)}$	$H_{(g)}$	$H_{(f)}$	$H_{(e)}$	$H_{(a)}$	$H_{(b)}$	R′	R″	R	$J_{g,f}$	$J_{a,b}$	$J_{a,c}$	$J_{a,d}$	$J_{b,c}$	$J_{b,d}$
a	CH_3	H	H	5.50	3.87	3.89	6.28	6.62	8.72	8.72	8.72	6.31	3.0	7.0	7.0	7.0		
b	CH_3	CH_3	CH_3	5.49	3.88	3.92	6.31	6.97	8.15	9.09	9.09	6.34	3.0	5.5			6.5	6.5
c	CH_3	H	C_6H_5	5.52	3.88	4.00	6.32	6.52	7.10	7.10	2.82	6.42	3.0	7.0	7.0			
d	CH_3	H	$-SCH_2-C_6H_5$	5.52	3.88	3.92	6.31	6.57	7.33	7.33	6.37 2.76	6.37	3.0	6.0	6.0			
e	$-C(CH_3)_3$	H	$-S-C(CH_3)_3$	5.49	3.85	3.87	6.23	6.63	7.21	7.21	8.72	8.53	3.0	6.0	6.0			
f	CH_3	H	OH	5.50	*ca.* 3.85		*ca.* 6.3	*ca.* 6.5	*ca.* 6.7		*ca.* 3.8	6.30						

Table 3. NMR spectra.



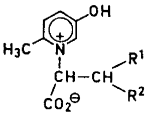
Comp. III	Solv.	Substituents		Chemical shift in τ values								Coupling constant in cps							
		R′	R″	CH_3	H_2	H_4	H_5	$H_{(a)}$	$H_{(b)}$	R′	R″	$J_{2,4}$	$J_{2,5}$	$J_{4,5}$	$J_{a,b}$	$J_{a,c}$	$J_{a,d}$	$F_{b,c}$	$F_{b,d}$
a	TFA	H	H	7.13	1.57	1.95	2.20	4.15	7.85	7.85	7.85	2.5	0	9.0	7.0	7.0	7.0		
b	»	CH_3	CH_3	7.12	1.39	1.94	2.18	4.77	ca. 7.3	8.65	9.07	2.5	0	9.0	10.5			6.5	6.5
c	»	H	C_6H_5	7.55	1.63	2.02	2.42	4.04	5.96	6.45	*ca.* 2.8	2.5	0	9.0	4.5	10.5		14.5	
d	»	H	$-SCH_2-C_6H_5$	7.45	1.75	1.97	2.32	4.50	6.28	6.58	2.65 6.15	2.5	0	9.0	4.7	9.5		15.0	
e	»	H	$-SH$	7.07	1.58	1.93	2.17	*ca.* 3.9	*ca.* 6.0	*ca.* 6.0	—	2.0	0	9.0					
e	D_2O	H	$-SH$	7.22	1.68	—	—	*ca.* 4.2	*ca.* 6.2	*ca.* 6.2	—								
f	»	H	$-OH$	7.31	1.51	—	—	4.50	5.62	5.62	—	2.5	0	9.0	6.0	6.0			

placed closely together as on the neighbouring carbon atoms in IIIc and IIId, strong preference for a certain conformation would be expected, most likely a conformation in which the large groups are *anti*. With substituents on the β-carbon which are not very space-requiring, *e.g.* hydroxyl or mercapto groups, no special conformation is preferred so that the methylene protons resonate in a doublet as in the furfurylamino acid esters (V). The same tendency for a preferred conformation is seen in the case of the valine derivative (IIIb), while in the furfuryl amino acid derivative (Vb) the methyl groups have the same chemical shift.

The aromatic protons in III are found in an ABX system. The 2-proton (1.6 τ) is coupled ($J = 2.5$ cps) to the lower field proton of the AB part (2.0 τ) which therefore is the 4-proton. The coupling with the 5-proton is negligible.

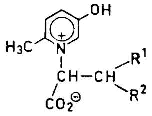
The products from the alkylamino acids (Va, Vb) are dextrorotary while the aryl amino acid (Vc) gives a strongly levorotatory product. The magnitude of the rotation increases sharply on going from aqueous acid to pure water solution, but the sign of the rotation remains the same (IIIa). The pyridinium oxides are optically stable on heating in dilute hydrochloric acid but not in dilute sodium hydroxide since the methine proton on the asymmetric carbon is readily abstracted, resulting in racemisation. However, the S-benzyl derivative (IIId) obtained was optically inactive. If the chemical shifts of the methine protons ($H_{(a)}$) in III (Table 3) are used as a rough guide to the relative acidities of these protons, it is seen that the proton in IIId is less acidic than in most cases. This compound should therefore be as optically stable as the other derivatives prepared. A possible explanation would be a β-elimination followed by β-thiol addition process. But it cannot be excluded that the racemisation largely occurred during the formation of the furfuryl amino acid (Vd) since the optical reading found (Table 1) was surprisingly low.

Table 4. Optical rotations measured at 25°.



Compound III	Substituents		N HCl aq			H$_2$O		
	R^1	R^2	[α]$_D$	[α]$_{546}$	c=g/100 ml	[α]$_D$	[α]$_{546}$	c=g/100 ml
a	H	H	+14	+18	0.8	+69	+73	0.6
b	CH_3	CH_3	0	0		+79	+83	0.7
c	H	C_6H_5	−90	−104	1.0	–	–	
d	H	$-SCH_2-C_6H_5$	0	0		0	0	

Table 5. UV absorption.



Comp. III	Substituents		N HCl aq		N NaOH aq	
	R^1	R^2	λ	log ε	λ	log ε
a	H	H	300	3.79	332	3.75
b	CH_3	CH_3	302	3.83	334	3.76
c	H	C_6H_5	302	3.80	333	3.75
d	H	$-SCH_2-C_6H_5$	304	3.77	335	3.74
e	H	SH	302	3.78	333	3.75

The pyridinium 3-oxides are blue fluorescent in UV light and absorb strongly at about 300 and 330 mμ in acidic and alkaline solutions, respectively. The difference between the absorption maxima is the normal bathochromic shift on going from a phenol to a phenolate ion.

The IR spectra in KBr show the C—O stretching vibration at 1610—1620 cm^{-1} which shows that the zwitterion is present as a carboxylate. After protonation of this group, as in the salt of stronger acids, the C—O band is found at 1730 cm^{-1}, which is that of a monomer and parallels the behaviour of amino acids.

EXPERIMENTAL

Paper chromatography or TLC on silica gel in the systems BuOH:EtOH:NH_3:H_2O (4:1:2:1) and BuOH:HOAc:H_2O (100:22:50) have been used throughout in this work. The NMR spectra were recorded on a Varian A-60A spectrometer and the UV data on a Perkin-Elmer model 137-UV spectrophotometer.

O-*Acetyl*-D-*serine methyl ester hydrochloride.* A solution of D-serine methyl ester hydrochloride (4.0 g, 0.026 mole) in acetic acid (150 ml) and acetic anhydride (50 ml) was saturated with dry HCl gas at 0°. The solution was then allowed to reach room temperature, left overnight, evaporated at reduced pressure and the residue crystallized from methanol-ether, yield 4.2 g (83 %), m.p. 161°. (Found: C 35.98; H 6.22; N 7.12; Cl 18.03. Calc. for $C_6H_{11}NO_4 \cdot HCl$: C 36.22; H 6.08; N 7.04; Cl 17.97). NMR (pyridine-d_5) Singlets at 8.10 ($-CO-CH_3$) and 6.25 τ (OCH_3). Multiplet at ~5 τ (CH_2-CH-).

N-*5-Hydroxymethylfurfuryl*-L-*phenylalanine methyl ester (Vc).* A solution of L-phenylalanine methyl ester hydrochloride (5.66 g, 0.029 mole) in methanol (100 ml) was neutralized with methanolic sodium methoxide, the precipitated salt removed by filtration, 5-hydroxymethylfurfural (5.4 g, 0.043 mole) added, the pH adjusted to pH 9 with sodium methoxide, Raney-Ni (W 6, ~13 g) added and the mixture hydrogenated at atmospheric pressure and room temperature. The reaction required 3 h for completion. The catalyst was then removed by filtering through a bed of "Celite" and the filtrate evaporated. The residual oil was extracted with ether (200 ml), the ether extract washed well with water (saturated with sodium chloride) to remove any amino acid, excess furfural and 2,5-dihydroxymethylfuran formed. Evaporation of the dried ether solution left a yellowish oil; yield 6.2 g (76 %). The yield at pH 11—12 was 53 %, at pH 5 about 3 %. The oil could not easily be made to crystallize, but was found to be chromatographically homogeneous. (Found: C 66.18; H 6.71; N 4.84. Calc. for $C_{16}H_{19}NO_4$: C 66.45; H 6.57; N 4.84).

N-*5-Hydroxymethylfurfuryl*-L-*alanine methyl ester (Va).* Prepared as above in 75 % yield. (Found: C 56.31; H 7.16; N 6.41. Calc. for $C_{10}H_{15}NO_4$: C 56.30; H 7.04; N 6.57).

N-*5-Hydroxymethylfurfuryl*-L-*valine methyl ester (Vb).* The reaction mixture was hydrogenated as above for 4 h when another equivalent of 5-hydroxymethylfurfural was added and the hydrogenation continued for 16 h. Yield 5.0 g (30 %). (Found: C 59.12; H 7.61; N 5.71. Calc. for $C_{12}H_{19}NO_4$: C 59.72; H 7.88; N 5.81).

S-*Benzyl*-N-*5-hydroxymethylfurfuryl*-L-*cysteine methyl ester (Vd).* *S*-Benzyl-L-cysteine methyl ester hydrochloride (5.25 g, 0.02 mole) and 5-hydroxymethylfurfural (2.25 g, 0.02 mole) in methanol (150 ml), the pH having been adjusted to 7 with sodium methoxide, were hydrogenated over 5 % palladium on charcoal (6.0 g). After 5 h the reaction mixture was worked up as above; yield 3.2 g (77 %). (Found: C 60.72; H 5.94; N 4.12. Calc. for $C_{17}H_{21}NO_4S$: C 60.83; H 6.27; N 4.18).

N-*5-Hydroxymethylfurfuryl*-S-tert-*butyl*-L-*cysteine* tert-*butyl ester (Ve).* *S*-*tert*-Butyl-L(-cysteine *tert*-butyl ester [12] (13. 5 g, 0.04 mole) and 5-hydroxymethylfurfural (7.5 g. 0.06 mole) in methanol (300 ml), the pH having been adjusted to 8.7 with sodium methoxide, were hydrogenated over 5% palladium on charcoal (10.0 g) at atmospheric pressure until about 3/4th of the theoretical amount of hydrogen had been consumed. Another 5.0 g of the catalyst was added and the hydrogenation continued for a total of 6 h. The reaction mixture was worked up as above; yield 15.0 g (75 %) of a yellowish oil. For analysis part of this material was converted into its solid hydrochloride by leading dry HCl into an ethereal solution. Recrystallisation of the white solid precipitate from methanol-ether gave m.p. 140°., (Found: C 53.77; H 8.10; N 3.99; Cl 9.33. Calc. for $C_{17}H_{29}NO_4S \cdot HCl$: C 53.78; H 7.91; N 3.70; Cl 9.36).

O-*Acetyl*-N-*5-hydroxymethylfurfuryl*-D-*serine methyl ester (Vg).* *O*-Acetyl-D-serine methyl ester hydrochloride (6.0 g, 0.03 mole) and 5-hydroxymethylfurfural (7.5 g 0.06 mole) in methanol (200 ml) at pH 8.4 (NaOMe) were hydrogenated over Raney-Ni (W-6) until twice the theoretical volume had been absorbed. The reaction mixture was filtered, the filtrate evaporated, the residue dissolved in methanol (20 ml) and diluted with ether (200 ml), the ethereal solution washed well with water (NaCl), dried and evaporated leaving a yellowish oil; yield 4.1 g (50 %). Chromatography showed this material to be largely homogeneous and was used further in the acid rearrangement as such.

N-*5-Hydroxymethylfurfuryl*-L-*serine methyl ester (Vf).* The reaction carried out as for the serine derivative above yielded a product strongly contaminated by other components. However, this was used as such in the acid rearrangements belov.

L-*(+)-2-(3-Hydroxy-6-methylpyridinium)propionate (IIIa).* *N*-5-Hydroxymethylfurfuryl-L-alanine methyl ester (1.0 g, 0.005 mole) in N HCl (100 ml) was heated at 100° for 10 h. The reaction mixture was filtered, evaporated, the residue dissolved in water (30 ml), the pH adjusted to 3.5 with NaOH aq., the solution extracted with 90 % phenol (3 × 20 ml), the combined phenol extracts washed with water (20 ml), ether (150 ml) added, the separated water layer collected, the ether-phenol layer washed with water (2 × 20 ml), the combined water phases washed with ether (20 ml) to remove any phenol and freeze dried. Crystallisation of the residue from water-acetone gave a white solid (0.25 g, 30 %), m.p. 146°. (Found: C 59.94; H 6.29; N 7.68. Calc. for $C_9H_{11}NO_3$: C 59.68; H 6.08; N 7.69).

L-*(+)-2-(3-Hydroxy-6-methylpyridinium)-3,3-dimethylpropionate (IIIb)* was prepared as above in 20 % yield, m.p. 136°. (Found: C 58.41; H 7.32; N 6.24. Calc. for $C_{11}H_{15}NO_3 \cdot H_2O$: C 58.15; H 7.48; N 6.18). An attempt to remove the water of crystallisation by drying at 60° resulted in partial decarboxylation.

L-*(—)-2-(3-Hydroxy-6-methylpyridinium)-3-phenylpropionate (IIIc).* *N*-5-Hydroxymethylfurfuryl- -phenylalanine methyl ester (4.0 g, 0.016 mole) in N HCl (150 ml) was heated at 100° overnight. The reaction mixture was filtered, the filtrate evaporated, dissolved in acetone and evaporated thrice before an acetone solution (50 ml) was left in the cold. The white solid precipitate was recrystallised from methanol-acetone-ether; yield 1.36 g (30 %), m.p. 155–156°. (Found: C 61.15; H 5.43; N 4.82; Cl 11.89. Calc. for $C_{15}H_{15}NO_3 \cdot HCl$: C 61.40; H 5.46; N 4.78; Cl 12.09). The zwitterion was liberated by dissolution in a small volume of water and adjusting the pH to 3. The precipitated zwitterion had m.p. 224°.

2-(3-Hydroxy-6-methylpyridinium)-3-thiobenzylpropionate (IIId) was prepared as the phenylalanine derivative above in 15 % yield, m.p. 121°. (Found: C 56.85; H 5.42; N 4.21; Cl 10.36. Calc. for $C_{16}H_{17}NO_3S \cdot HCl$: C 56.61; H 5.31; N 4.13; Cl 10.46). The yellowish white zwitterion, liberated as above, melted at 116°, after crystallisation from water. The compound was optically inactive.

2-(3-Hydroxy-6-methylpyridinium)-3-mercaptopropionate (IIIe). a) 2-(3-Hydroxy-6-methylpyridinium)-3-thiobenzylpropionate (0.17 g, 0.0005 mole) was dissolved in acetic acid (25 ml) previously saturated with HBr and the solution left at room temperature for 3 days. The solution was then evaporated at reduced pressure, the residual oil dissolved in methanol (1 ml) and the solid hydrobromide precipitated with acetone-ether; yield 0.10 g (90 %), m.p. 110–113 (slow decomposition from about 60°). (Found: C 36.30; H 4.03; N 4.35. Calc. for $C_9H_{11}NO_3S \cdot HBr$: C 36.70; H 4.08; N 4.76).

b) *N*-5-Hydroxymethylfurfuryl-*S*-*tert*-butyl-L-cysteine *tert*-butyl ester (15.0 g, 0.044 mole) in 3 N HCl (500 ml) was heated at 100° overnight. The reaction mixture was then filtered, evaporated, the residue dried by dissolution twice in acetone followed by evaporation and the pyridinium product made to crystallize from acetone by ether addition. Chromatography showed this to be mainly the desired product but contaminated with the corresponding disulphide (XXVI) and the acrylate (XXIII) arisen by β-elimination.

The disulphide (XXVI) was readily formed by bubbling air through a weakly alkaline solution of the thiol.

2-(3-Hydroxy-6-methylpyridinium)-3-hydroxypropionate (IIIf); 2-(3-hydroxy-6-methylpyridinium)acrylate (XXIII). *O*-Acetyl-*N*-5-hydroxymethylfurfuryl D-serine methyl ester and the same derivative of serine (Vf) when subjected to acid catalysed rearrangements furnished a pyridinium-3-oxide product as determined by UV absorption with variable ratios of the two title compounds (by NMR and chromatography) depending on the conditions used.

Hydroxymethylation of N*-furfurylalanine methyl ester; 2-(3-hydroxy-6-methylpyridinium)propionate (IIIa).* An ice-cold solution of L-*N*-furfurylalanine methyl ester[17] (8.5 g, 0.046 mole) and 37 % formaldehyde (5.0 g) in 6 N HCl (50 ml) was added dropwise over 30 min to boiling 3 N HCl (20 ml). The reaction was kept at reflux for another hour and filtered. Chromatography showed a complex product mixture and UV absorption measured at 300 mμ showed that the yield of the pyridinium-3-oxide was about 20 %.

The Mannich reaction on alanine; 2-(3-hydroxy-6-methylpyridinium)propionate (IIIa). A solution of L-alanine (4.5 g, 0.05 mole) and 37 % formaldehyde (10 ml, 0.10 mole) in water (20 ml) was brought to pH 3 with conc. HCl and furfuryl alcohol (4.9 g, 0.05 mole) added dropwise over 15 min. The reaction mixture was left at room temperature for two days. Chromatography showed that the hydroxymethylfurfural first formed had been rearranged to the pyridinium-3-oxide. The reaction mixture was therefore added dropwise to boiling 2 N HCl (200 ml).

Chromatography and UV absorption showed that the yield of pyridinium compound was about 10 %.

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