Isolation, Structure, and Synthesis of Hermidin, a Chromogen from *Mercurialis perennis* L.[1]

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Hermidin has been isolated from *Mercurialis perennis* L. as a colourless, crystalline compound, shown by synthesis to be 5-hydroxy-4-methoxy-1-methylpyridine-2,6(1*H*,3*H*)-dione (**2**), which in aqueous solution exists as a dihydroxy-4-methoxy-1-methylpyridin-2(1*H*)-one (**3**) or (**4**). Oxidation of hermidin with nitric acid affords 4-methoxy-1-methylpyridine-2,3,6-trione (**16**). When equimolecular amounts of the latter and hermidin in aqueous solution are mixed an immediate deep blue solution containing an ion-radical (**17**) results. Oxidation of hermidin by air in methanolic solution gives 5,5'-dihydroxy-4,4'-dimethoxy-1,1'-dimethyl-3,3'-bipyridine-2,2',6,6'-(1*H*,1'*H*,3*H*,3'*H*)-tetraone (**18**), and in pyridine solution 4,4'-dimethoxy-1,1'-dimethyl-3,3'-bipyridine-2,2',5,5',6,6'-hexaone (**23**). Knackmuss' conclusions on the oxidation of 3,6-dihydroxy-4-methylpyridin-2(1*H*)-one (**26**) have been re-interpreted. 3,4-Dihydroxy-5-methoxy-1-methylpyridin-2(1*H*)-one (**7**) has been synthesized.

When the green plant *Mercurialis perennis* L. (belonging to the Euphorbiaceae) is allowed to dry it assumes an inky-blue, almost black colour. In 1893 Molisch[2] stated that this colour was not due to indigo as it turned red when treated with acid. The literature contains references to animals fed on the plant producing red urine and excreta, and the plant is said to have diuretic action[3] and to be poisonous. Also, colourless stems which are bruised or cut can develop transient blue or yellow colour.

The pigment was investigated by Haas and Hill[4] in collaboration with Cannan[5] and, although they discovered nothing about the structure of the pigment or the colourless chromogen from which it was formed, they found that the chromogen (a powerful reducing agent) was most abundant in the young and vigorously growing plant. From this source they prepared solutions by extraction (1 h at 45 °C under nitrogen) of fresh plant material with water containing a small amount of chloroform. These extracts had a great avidity for oxygen and oxidation occurred in two stages, each being accompanied by the absorption of an equal volume of oxygen. The first stage resulted in the formation of a fugitive blue compound which on further oxidation gave rise to a more permanent yellow-brown compound, both stages being independent of enzyme action. The fugitive blue compound was different from the relatively stable blue compound produced by drying the plant material. They named the colourless chromogen hermidin, the fugitive blue compound cyanohermidin, and the yellow-brown compound chrysohermidin. Cyanohermidin could be reduced back to hermidin by sodium dithionite, and chrysohermidin could be reduced by aluminium amalgam immediately after its formation, although after being kept for 2 h it became incapable of being reduced. From the results of potentiometric titrations Cannan[5] concluded that the changes could be summarised:

$$\underset{\text{(colourless)}}{\text{Hermidin}} \underset{+2H}{\overset{-2H}{\rightleftharpoons}} \underset{\text{(fugitive blue)}}{\text{Cyanohermidin}} \underset{+2H}{\overset{-2H}{\rightleftharpoons}}$$

$$\text{Oxidant} \rightleftharpoons \underset{\text{(yellow-brown)}}{\text{Chrysohermidin}}$$

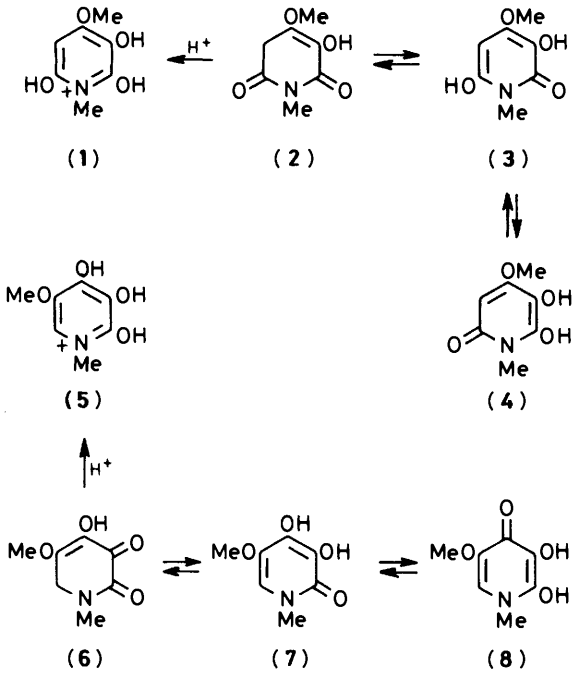
He thought that chrysohermidin was formed from the true oxidant of cyanohermidin by a change which was not oxidative, but whose velocity was a function of pH. Haas and Hill[4a] concluded that both blue compounds were produced from the same precursor and that if the chromogen is converted into the transient blue compound it cannot form the permanent blue substance.

The more stable blue compound obtained by drying the young plant material changes spontaneously with time, or more rapidly when an aqueous solution of it is warmed, into a red pigment from which were separated a series of pigments, said to be glycosides containing C, H, N, and S,[6] although the glycosidic nature was later denied.[3] Gedeon and Mayer[3] claimed to have obtained a red fraction containing Fe, S, and N, degradation of which with hydrogen bromide in acetic acid yielded a solution, the absorption spectrum of which in the visible region was porphyrin-like. However evidence for the pigment being a porphyrin seems very slender.

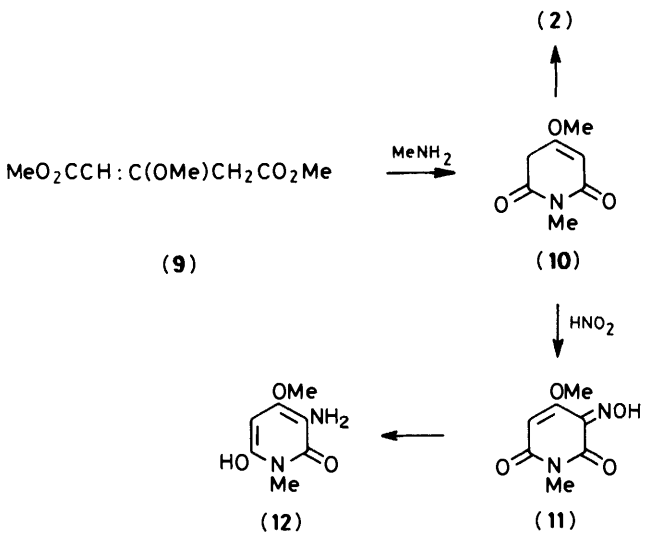
Mercurialis perennis is known to contain methylamine and an amine transaminase.[7]

Isolation of a Chromogen (*Compound* **A**).—An aqueous extract of *Mercurialis perennis* prepared as described by Haas and Hill[4b] was extracted with chloroform in the presence of sodium dithionite. Evaporation of the dried chloroform extract yielded a product which after rapid recrystallisation from chloroform–ether afforded colourless crystals of almost pure compound **A**. Further purification was effected by rapid chromatography and sublimation. The same compound, in modest yield, was obtained from *Mercurialis annua* L., collected in late summer.

Structure of Compound **A**.—Methylamine was evolved when compound **A** was boiled with M-NaOH under nitrogen. Mass spectrometry and analysis established the molecular formula of **A** as $C_7H_9NO_4$ and this together with the n.m.r. spectrum suggested two possible structures for the crystals: 5-hydroxy-4-methoxy-1-methylpyridine-2,6(1*H*,3*H*)-dione (**2**) or 4-hydroxy-5-methoxy-1-methylpyridine-2,3(1*H*,6*H*)-dione (**6**). In aqueous solution these probably exist in tautomeric equilibrium with 3,6-dihydroxy-4-methoxy-1-methylpyridin-2(1*H*)-one (**3**) and (**4**) or with 3,4-dihydroxy-5-methoxy-1-methylpyridin-2(1*H*)-one (**7**) and (**8**), and in trifluoroacetic acid as the pyridinium salts (**1**) or (**5**), respectively. Although the formation of methylamine on hydrolysis favoured structure (**2**), the alternative (**6**) was considered seriously because when **A** was treated with phenylhydrazine it yielded a crystalline product, the electronic spectrum of which resembled that of dehydroascorbic acid osazone. It was therefore decided to synthesize both (**2**) and (**6**); compound **A** proved identical with the former.



Synthesis of Compound **A**.—Treatment of dimethyl 2-oxopropane-1,3-dicarboxylate with methanol in the presence of trimethyl orthoformate and sulphuric acid yielded dimethyl 2-methoxypropene-1,3-dicarboxylate (**9**) (*cf.* ref. 8), which reacted with an equimolecular amount of methylamine in aqueous solution to afford 4-methoxy-1-methylpyridine-2,6(1*H*,3*H*)-

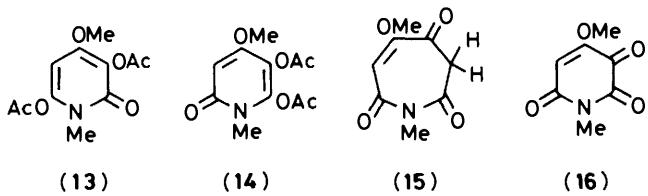


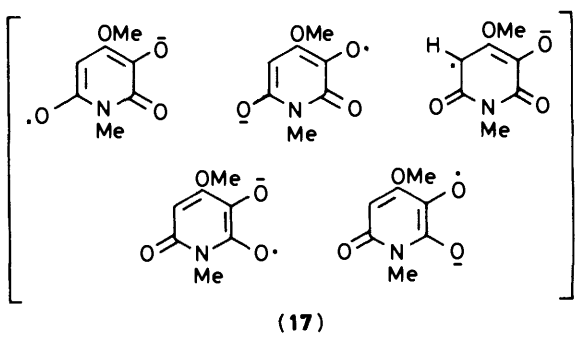
dione (**10**). The latter was converted in modest yield by Elbs peroxydisulphate oxidation into compound (**2**) (*cf.* ref. 9), identical with **A** isolated from *Mercurialis perennis*. Later what appears to be a better synthesis was found, although this has not been worked out in detail. Treatment of compound (**10**) with an excess of nitrous acid yielded 4-methoxy-1-methyl-3-oximinopyridine-2,6(1*H*,3*H*)-dione (**11**) which was reduced by tin in the presence of concentrated hydrochloric acid to afford a hydrochloride of 3-amino-6-hydroxy-4-methoxy-1-methylpyridin-2(1*H*)-one (**12**). Surprisingly, attempts to recrystallise the latter from aqueous hydrochloric acid yielded the hydrochloride of **A**, *i.e.* 2,3,6-trihydroxy-4-methoxy-1-methylpyridinium (**1**) chloride.

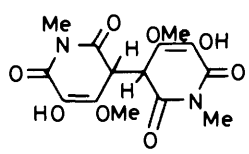
Derivatives of Compound **A**.—Treatment of compound **A** with a mixture of acetic anhydride and trifluoroacetic acid afforded a diacetyl derivative, either 3,6-diacetoxy-4-methoxy-1-methylpyridin-2(1*H*)-one (**13**) or 5,6-diacetoxy-4-methoxy-1-methylpyridin-2(1*H*)-one (**14**). However reaction of **A** with acetic anhydride in pyridine yielded a different product, apparently an acetoxyacetylhydroxy-4-methoxy-1-methylpyridin-2(1*H*)-one.

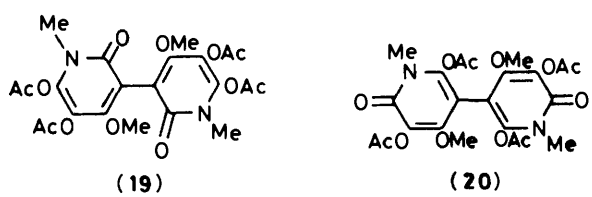
Reaction of Compound **A** with diazomethane afforded two products. The first analysed as $C_8H_9NO_4$ and is tentatively assigned the azepine structure (**15**) whilst the second analysed as $C_8H_{11}NO_4$ although the spectroscopic evidence suggested a dimeric structure.

Oxidation Products of Compound **A**.—(*a*) When compound **A** was treated with concentrated nitric acid at −40 °C it yielded 4-methoxy-1-methylpyridine-2,3,6-trione (**16**). The same product was also obtained from **A** in phosphate buffer at pH 7.2 by rapid oxidation with $K_3[Fe(CN)_6]$ or by oxidation with 1,4-benzoquinone.









When equimolecular amounts of **A** and (**16**) were mixed in phosphate buffer of pH 7.1 an immediate deep blue colour was produced. Compounds **A** and (**16**) can be regarded as analogous to a quinol and a quinone, respectively, and the blue compound might then be a semiquinone-like radical-anion (**17**); e.s.r.

evidence consistent with the latter structure has been presented by Forrester.[10]

(*b*) When a solution of **A** in chloroform was kept for several hours at room temperature, colourless crystals of compound **B** separated. The same product was obtained more rapidly by warming a solution of compound **A** in methanol. Compound **B** has a very low solubility in common organic solvents and it was therefore difficult to obtain a satisfactory n.m.r. spectrum of it. A spectrum of **B** was obtained in $(CD_3)_2SO$ although it was not clear whether the splitting of the peaks was a result of tautomerism or of partial oxidation.

The molecular formula of **B** was $C_{14}H_{16}N_2O_8$ and its mass spectrum was like that of **A**, but with an additional peak at *m/z* 169, probably indicating thermal decomposition to give **A** and the trione (**16**). The i.r. spectrum of **B** was very similar to that of **A** except that it had peaks at 3 400, 1 200, 835, and 760 cm^{-1}, whereas **A** had peaks at 3 290 and 765 cm^{-1}. Compound **B** was formulated as 5,5′-dihydroxy-4,4′-dimethoxy-1,1′-dimethyl-3,3′-bipyridine-2,2′,6,6′(1*H*,1′*H*,3*H*,3′*H*)-tetraone (**18**) or tautomer.

When **B** was treated with the mixed acetic trifluoroacetic anhydride it yielded a more soluble acetyl derivative formulated as either 5,5′,6,6′-tetra-acetoxy-4,4′-dimethoxy-1,1′-dimethyl-3,3′-bipyridine-2,2′(1*H*,1′*H*)-dione (**19**) or 2,2′,5,5′-tetra-acetoxy-4,4′-dimethoxy-1,1′-dimethyl-3,3′-bipyridine-6,6′(1*H*,1′*H*)-dione (**20**).

From the n.m.r. spectrum it appears that in solution in trifluoroacetic acid compound **B** disproportionates to give a mixture of **A** and (**16**); a small amount of compound (**23**) is also formed. This is reminiscent of the *meso*- and ± 1-photoreductive dimers of 5,6-dihydro-3,5,5-trimethyl-1,4-oxazine-2-one which in solution exist in equilibrium with the radical 5,6-dihydro-3,5,5-trimethyl-2-oxo-1,4-oxazin-3-yl.[11] Oxidation of **B** with nitric acid yielded compound (**16**). When compound **B** was heated with 2,2′-azo(2-methylpropionitrile) in acetic acid a product was obtained formulated as 3-(2-cyanoisopropan-2-yl)-5-hydroxy-4-methoxy-1-methylpyridine-2,6-(1*H*,3*H*)-dione (**21**) or a tautomer. Treatment of **B** with aqueous sodium dithionite resulted in reduction back to **A**.

(*c*) Compound **B** dissolved in dry pyridine under nitrogen to give a colourless solution which when exposed to air rapidly became blue, then yellow, and finally red, and from which a yellow compound formulated as 4,4′-dimethoxy-1,1′-dimethyl-3,3′-bipyridine-2,2′,5,5′,6,6′-hexaone (**23**) was isolated. The latter was also obtained similarly from compound **A**. The n.m.r. spectrum of this yellow compound consisted of two singlets of equal area at δ 3.35 and 4.15. Its mass spectrum showed a molecular ion, $C_{14}H_{12}N_2O_8$, at *m/z* 336, together with a larger peak at 321 and a small peak at 338. Benzoquinones frequently show $M + 2$ peaks in their mass spectra.[12]

(*d*) When compound **B** was oxidised with ceric ammonium nitrate in acetic acid it afforded a second yellow product, the i.r. spectrum of which was very similar to that of compound (**23**), but the *m/z* 336 and 338 peaks were approximately equal in height in the mass spectrum which otherwise resembled that of (**23**). The i.r. spectra of both yellow products contained peaks at 1 715 and 1 660 cm^{-1}. Kuhn, Bauer, and Knackmuss[13] described compounds (**24**) and (**25**) as being yellow and red, with i.r. bands at 1 750 and 1 670 cm^{-1}, respectively. However, the n.m.r. spectrum of this oxidation product of **B** was strikingly different from that of (**23**) and was consistent with that of a solution containing a mixture of 1 mol equiv. of (**23**) and 2 mol equiv. of compound (**16**). It therefore seems that the crystals consist of a quinhydrone-like complex of equimolecular amounts of (**23**) and (**22**) and that in solution in $CDCl_3$ or CF_3CO_2H this decomposes to yield a mixture of (**23**) and (**16**).

(*e*) As described by Haas and Hill, when an aqueous extract of *Mercurialis perennis* was shaken with air it became, during the course of minutes, first deep blue, then green, and finally, yellow-brown. Extraction of the final solution with chloroform afforded a product indistinguishable from that obtained from the oxidation of compound **B** by ceric ammonium nitrate. However, the chloroform extract although initially only very pale yellow became deep yellow-brown during drying with sodium sulphate.

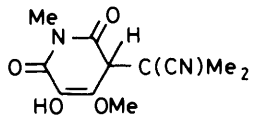
In titrating extracts of *Mercurialis perennis* at pH 3.99, Cannan[5] thought that he was titrating the oxidation of hermidin to cyanohermidin. In the present study when compound **A** was titrated at pH 4 a one-step curve was obtained like that of Cannan and, in fact, this represented just under 2 equiv. of $K_3[Fe(CN)_6]$ per mol of **A** so that the product must be (**16**) (Figure 1). Only a very pale blue colour developed even at the mid-point, indicating a very low concentration of (**17**).

Carrying out the titration at pH 7.61 Cannan obtained two steps on the curve which he thought represented two 2-electron steps:

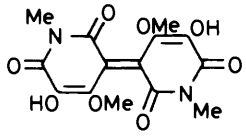
$$\text{Hermidin} \xrightarrow{-2H} \text{Cyanohermidin} \xrightarrow{-2H} \text{Chrysohermidin}$$

This titration and also the titration of **A** under the same conditions, were repeated. Considering that the aqueous extract of *M. perennis* which was titrated must have contained many compounds other than hermidin, the curve obtained fits reasonably well with that obtained by titrating compound **A** under the same conditions, although the concentration of **A** in the plant extract was lower and unknown. The titration of **A** at pH 7.6 shows a first step when rather less than 2 equiv. of oxidising agent per mol of **A** have been added, followed by a second step after a further *ca.* 0.5 equiv.

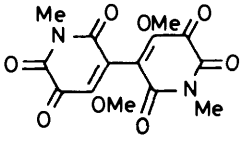
Presumably compound **A** is initially oxidised to (**16**) which at pH 4 reacts with **A** only extremely slowly [because **A** exists as (**1**)] so in the titration an almost quantitative conversion of **A** into (**16**) is achieved without dimerisation. However at pH 7.6 compound (**16**) can react with **A** [existing as (**3**) or (**4**)] to give **B** albeit at a rate lower than the rate of oxidation of **A** to (**16**) so at the first potential step the products consist of rather less than 1 mol equiv. of (**16**) and a relatively small amount of **B**. The second step represents the oxidation of compound **B** to (**22**) and possibly to (**23**). Assuming that the oxidation of *M. perennis* extract by air proceeds similarly the initial pale yellow chloro-



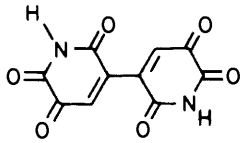
(**21**)



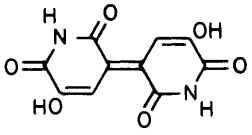
(**22**)



(**23**)



(**24**)



(**25**)

form extract will contain (**16**) together with a small amount of (**22**) and perhaps (**23**). Theoreticallly, dimerisation of (**16**) to give (**22**) involves neither oxidation nor reduction; it is conceivable that some redox reaction may initiate the dimerisation leading to the formation of the quinhydrone-like (**22**)—(**23**) and deepening of colour.

The titration is relatively slow so considerable amounts of dimeric products arise, whereas when the oxidation is carried out rapidly or under strongly acidic conditions the main product isolated is (**16**), *e.g.* see experimental details of oxidation of **A** to (**16**) by nitric acid, 1,4-benzoquinone, or $K_3[Fe(CN)_6]$.

Oxidation of 3,6-*Dihydroxy*-4-*methylpyridin*-2(1H)-*one* (**26**).—Knackmuss has studied azaquinones [14a] extensively and has suggested [14b] that the course of oxidation of 3,6-dihydroxy-4-methylpyridin-2(1H)-one (**26**) (which is related structurally to **A**) could be explained as shown in Scheme 1.

The initially formed colourless 2,2′,5,5′-tetrahydroxy-4,4′-dimethyl-3,3′-bipyridine-6,6′(1*H*,1*H*′)-dione (**27**) was supposed to pass through the blue-violet (**28**) to form the yellow 4,4′-dimethyl-3,3′-bipyridine-2,2′,5,5′,6,6′-hexaone (**29**). However he failed to isolate the blue-violet intermediate, which if it had structure (**28**) should be extractable by chloroform. And in any case, Kuhn, Bauer, and Knackmuss [13] had earlier described compound (**25**) as red crystals giving a yellow-brown solution in *N*,*N*-dimethylformamide. In agreement with Knackmuss, atmospheric oxidation of (**26**) in water yielded a colourless, dimeric compound (**27**) and oxidation of this with nitric acid afforded a yellow compound which Knackmuss stated to be $C_{12}H_8N_2O_6$, *i.e.* the compound which he represented as (**29**). However, according to the mass spectrum, the yellow compound which was obtained in this study was $C_{12}H_{10}N_2O_6$, *i.e.* it has the structure (**28**) which he attributed to the blue-violet compound. In the opinion of this author the blue-violet compound must be analogous to a semiquinone.

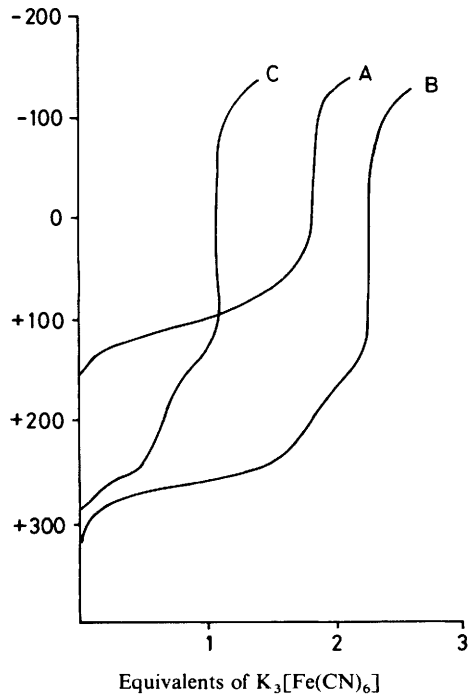


Figure 1. A, compound A at pH 4.0; B, compound A at pH 7.6; C, *Mercurialis perennis* extract at pH 7.6

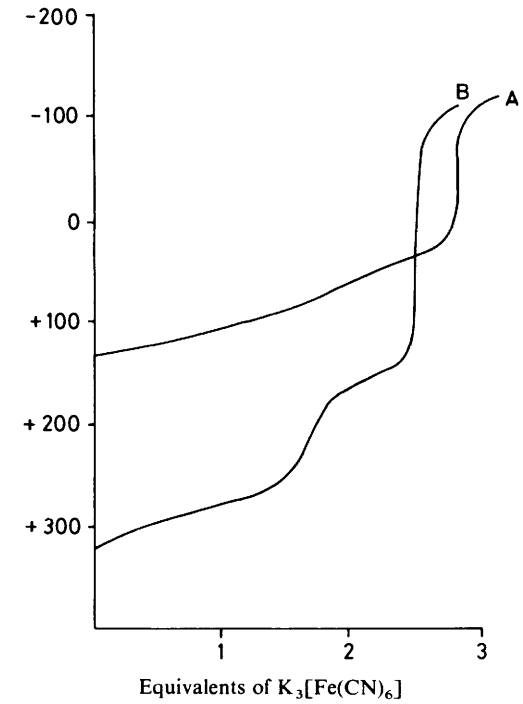
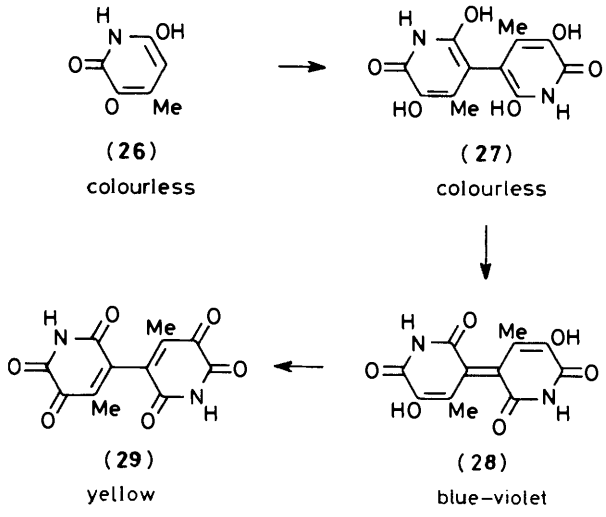
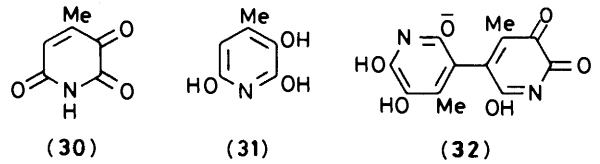


Figure 2. 3,6-Dihydroxy-4-methylpyridin-2(1*H*)-one at A, pH 4.0, and B, pH 7.6



Scheme 1.



Knackmuss' scheme was based partly on potentiometric titrations with $K_3[Fe(CN)_6]$ at pH 9.2. These titrations were repeated on (**26**) at pH 7.6 and 4 (Figure 2). At pH 7.6 a curve was obtained similar to that of Knackmuss, *i.e.* the first step after just under 2 equiv., and the second after just under 3 equiv. of $K_3[Fe(CN)_6]$ per mol of (**26**). However, at pH 4 no step

occurred until 3 equiv. of oxidising agent had been added, the solution remaining deep blue throughout up to the end-point when it became yellow.

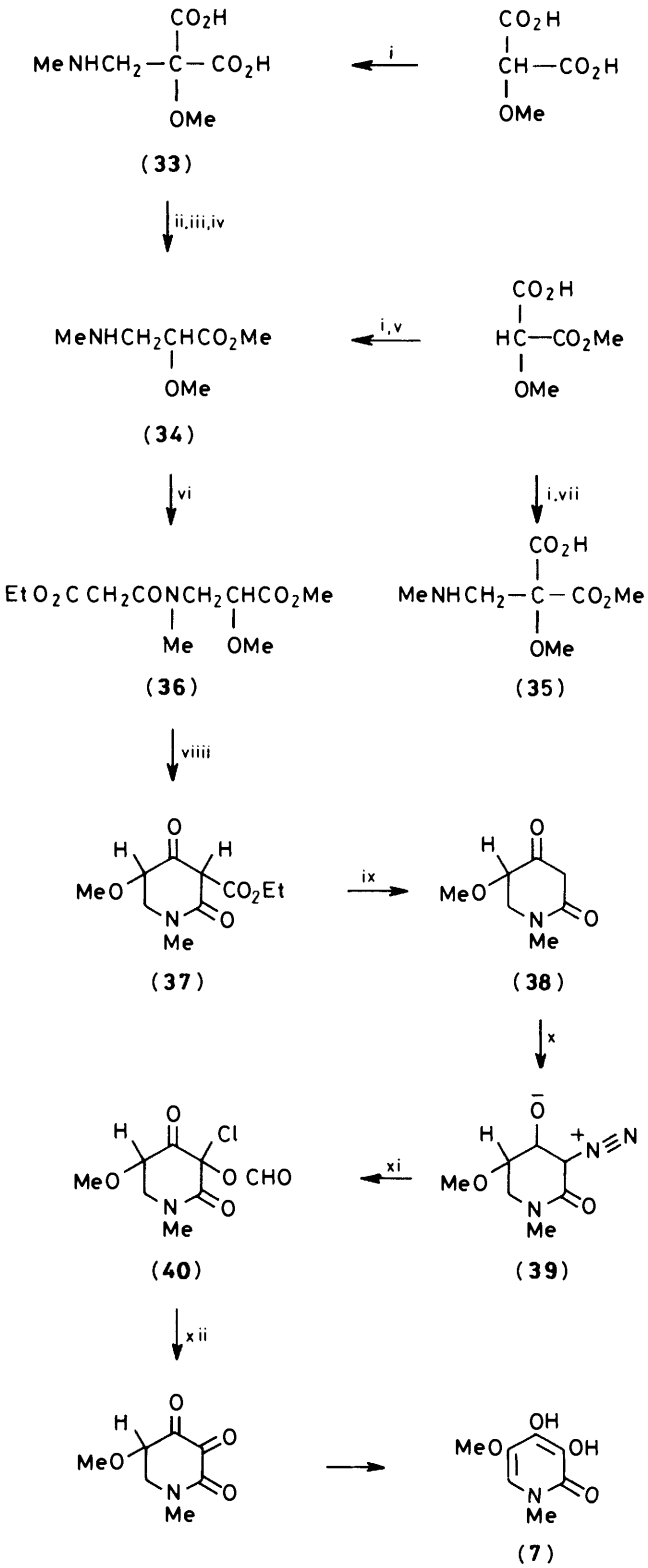
These results can still be explained on the basis of Scheme 1, but accepting that compound (**28**) is yellow and not blue-violet and that the primary step is the oxidation of (**26**) to give 4-methylpyridine-2,3,6-trione (**30**), which can then react with (**26**) to give (**27**) in an acid-catalysed reaction [which is rapid at pH 4 because at this pH the compound would occur predominantly as 4-methylpyridine-2,3,6-triol (**31**), rather than as (**26**), which will be more favourable for reaction with (**30**)], whereas in the case of the oxidation of **A** the latter would probably exist mainly as (**1**) [or possibly (**3**)], which would be less favourable for reaction with (**16**). The resulting (**27**) would probably exist in acid solution largely in a hexahydroxy form, favourable for oxidation, so this might be oxidised to (**29**) without a further step on the curve. At pH 7.2 the rate of dimerisation would be much lower, so that the situation would resemble that in the oxidation of **A**, *i.e.* after the addition of 2 equiv. of oxidant the product would consist mainly of (**30**) together with a little (**27**) and (**28**). Further oxidation would lead to (**29**). In Knackmuss' titration at pH 9.2 the first 2-equiv. step presumably represents the formation of (**28**), which in alkaline solution would exist as an anion, stabilised by adopting structure (**32**) (or similar tautomer). The final 1-equiv. step would represent oxidation of (**32**) to (**29**).

Conclusion.—To summarise, it is suggested that the chromogen present in the colourless aqueous extract of *Mercurialis perennis* prepared as described by Haas and Hill is compound **A** and that on oxidation by air or $K_3[Fe(CN)_6]$ the blue transient colour is due to a semiquinone-like (**17**), and that at the moment when the solution turns yellow it contains compounds (**16**), (**22**), and (**23**).

The question remains as to whether compound **A** is what Haas and Hill called hermidin. Although there is no doubt that **A** is the chromogen present in an extract prepared according to these authors, the possibility remains that in the living plant there is some other compound which is converted into **A** during the course of extraction. It was, however, found possible to obtain compound **A** by merely homogenising the fresh plant in cold sodium acetate–sodium dithionite solution for 1 min and extracting immediately with chloroform.

Synthesis of 3,4-*Dihydroxy*-5-*methoxy*-1-*methylpyridin*-2(1H)-*one* (**7**).—A Mannich reaction of methoxymalonic acid with methylamine and methanal gave methoxy(methylaminomethyl)malonic acid (**33**), which when decarboxylated and then treated with thionyl chloride followed by methanol, yielded methyl 2-methoxy-3-methylaminopropanoate (**34**). However, the latter was better obtained by a Mannich reaction on monomethyl methoxymalonate, followed by treatment with methanolic hydrogen chloride. In the latter Mannich reaction when a high concentration of acetic acid was present methyl methoxy(methylaminomethyl)malonate (**35**) was formed.

Reaction of the base (**34**) with ethoxycarbonylacetyl chloride yielded the amide (**36**), which underwent a Dieckmann reaction to give the oxo ester (**37**). The latter was hydrolysed and decarboxylated to 5-methoxy-1-methylpiperidine-2,4-dione (**38**) in poor yield by boiling in 10% acetic acid (*cf.* ref. 15) because of the tendency for the 1,3-dicarbonyl product to undergo self-condensation. Later, in the course of work on similar 5-ring compounds a mild method was discovered which may possibly be applicable here, *e.g.* in 3-ethoxycarbonyl-4-hydroxy-1,5-dimethyl-2,5-dihydropyrrol-2-one the CO_2Et group is replaced by H by boiling under reflux for 1 h with t-butanol containing 1% of water (the completion of the reaction is seen by the disappearance of colouration with $FeCl_3$). In the latter case this method was found to be much more convenient and to give higher yields than that used by Mulholland, Foster, and Haydock.[16]



Reagents: i, $MeNH_2$–HCHO; ii, H_2O/heat; iii, $SOCl_2$; iv, MeOH; v, MeOH–HCl; vi, CO_2EtCH_2COCl, vii, $MeCO_2H$; viii, Na–xylene; ix, 10% $MeCO_2H$; x, toluene-*p*-sulphonyl azide–NEt_3; xi, HCO_2H–Bu^tOCl; xii, sublimation.

Reaction of the dione (**38**) with toluene-*p*-sulphonyl azide in

the presence of triethylamine afforded 3-diazo-5-methoxy-1-methylpiperidine-2,4-dione (**39**) in very low yield (*cf.* ref. 17). Replacement of the azide by a resin to which a sulphonyl azide group was attached [18] led to some increase in yield. Reaction of a solution of the diazo ketone in formic acid with t-butyl hypochlorite gave a product (**40**) which sublimed with decomposition to afford 3,4-dihydroxy-5-methoxy-1-methylpyridin-2(1*H*)-one (**7**) (*cf.* ref. 17). Unlike its isomer (**2**) compound (**7**) did not yield blue products on oxidation, and, moreover, the crystals appeared to consist of (**7**) rather than (**6**).

Experimental

Except where stated otherwise all ^{1}H n.m.r. spectra were measured at 60 MHz in $CDCl_3$, all electronic spectra in methanol, and all i.r. spectra as pressed KBr discs; all chromatography was carried out on Merck silica using chloroform as eluant. All evaporations were under reduced pressure. Liquids were dried with Na_2SO_4 and solids *in vacuo* in a vacuum desiccator. Melting points are uncorrected.

Isolation of Compound **A** *from* Mercurialis perennis.—The plant was collected in Northumberland at various times during the period February—April (or May in upland areas). However, material collected in March—April gave the highest productivity of **A**. When the plant was growing in deep leaf-mould it often possessed long lengths of colourless stem not exposed to light; these colourless portions of stem were a good source of **A**. However, although the parts of the plant exposed to light were green, the presence of chlorophyll proved no obstacle so far as isolation of **A** was concerned. For most extractions therefore no attempt was made to separate the colourless and green parts of the stem. However, the leafy tips were cut off as well as any lower leaves, and these were for convenience extracted separately from the stems. Where necessary any adhering soil was washed off rapidly with cold water, and the stems were blotted on paper to dry them rapidly. Extraction was usually carried out on the day following collection, although reasonably good results were obtained if the plant material was stored in a Polythene bag in a refrigerator for up to 3 days.

A number of stems were held together in a bundle and rapidly cut up by scissors into lengths of *ca.* 8 mm. The cut material (220 g) was added as rapidly as possible to cold air-free distilled water (250 ml) containing chloroform (5 ml) contained in a glass cylinder (13 cm in length, 8 cm in diameter) sealed to a B34 cone. During this time nitrogen was passed into the cylinder. The cone was then connected to a B34 socket, to which was sealed a coarse fritted-glass filter and a short, wide tube provided with a gas-delivery tube and another tube leading through a 2-holed rubber stopper inserted in the neck of an inverted separating-funnel (500 ml). The other hole of the rubber stopper carried a gas-outlet tube. Oxygen-free nitrogen was passed into the gas-delivery tube and thence through the separating-funnel and out through the outlet tube. The cylinder was heated in a water-bath at 45 °C for 1 h. The apparatus was then carefully inverted so that the aqueous extract was filtered into the separating-funnel, still in an atmosphere of nitrogen. The pH of this solution was *ca.* 6. The rubber stopper was then raised slightly out of the neck of the separating-funnel, a narrow funnel was inserted into the separating-funnel, and a solution of sodium dithionite (1.5 g) in water (5 ml) was added, followed by chloroform (70 ml). The separating-funnel was removed from the rest of the apparatus and was then stoppered and shaken, and the contents were filtered through HiFlo Supercel. The chloroform layer was rapidly separated, briefly dried over a mixture of sodium sulphate and sodium dithionite, and filtered; the chloroform was then rapidly removed at room temperature using a rotary evaporator, leaving a residue of crude **A**. Further sodium dithionite (1 g) was added to the aqueous layer and re-extraction with chloroform yielded further **A**. Later extractions afforded purer **A** than the first extraction. However, it was found more convenient to simply extract the aqueous solution with chloroform in a continuous extractor for several hours.

The compound was purified by dissolving it rapidly in a minimum volume of hot chloroform, diluting the solution with ether, and allowing it to cool, when colourless crystals of **A** separated; these were filtered off, washed with ether, and dried. In the case of vigorously growing plants collected in March—April the yield of **A** obtained from 220 g stem (250 ml water) or from 120 g green tops (250 ml water) could be as high as 0.3 g or 0.35 g, respectively.

In t.l.c. on silica (chloroform) this material gave a number of spots of various colours (yellow, red, purple, brown). However, **A** could be purified without too much loss by rapid chromatography on a short column of silica, using chloroform as eluant. The almost pure material could also be purifed by sublimation at 110 °C/0.01 mmHg, and the analytical sample finally recrystallised from chloroform–ether. When heated the crystals decomposed without any sharp melting point (Found: C, 49.2; H, 5.2; N, 8.25%; $M^{\bullet+}$, 171.0534. $C_7H_9NO_4$ requires C, 49.1; H, 5.3; N, 8.2%; $M^{\bullet+}$, 171.0532); $\nu_{max.}$ 3 290, 1 710—1 635, and 765 $c,^{-1}$; $\lambda_{max.}$ (freshly prepared) 277 nm, rapidly shifting to 266 nm with slight increase in intensity; δ 3.2 (3 H, s, NMe), 3.5 (2 H, s, CH_2), 4.05 (3 H, s, OMe), and 5.75 (1 H, br s, OH); δ(CF_3CO_2H) 3.9 (3 H, s, NMe), 4.1 (3 H, s, OMe), and 6.5 (1 H, s, CH). The crystals had powerful sternutatory properties.

Hydrolysis of **A**.—A solution of **A** (36 mg) in M NaOH (2 ml) was boiled under reflux while nitrogen was passed first through this then through 2M-HCl. The acid solution was evaporated to dryness yielding a white solid residue (4 mg) identified by mass spectrum as methylammonium chloride.

Dimethyl 2-Methoxypropene-1,3-dicarboxylate (**9**).—A mixture of dimethyl 2-oxopropane-1,3-dicarboxylate (11.8 ml), trimethyl orthoformate (12 ml), methanol (12.5 ml), and concentrated sulphuric acid (0.4 ml) was left overnight at room temperature and then boiled under reflux for 6 h. The cooled solution was diluted with ether, washed with aqueous sodium hydrogen carbonate, dried, and distilled to yield the product (14.5 g), b.p. 135 °C/1 mmHg; δ 3.6 (3 OMe), 3.85 (s, CH_2), and 5.1 (s, CH).

4-Methoxy-1-methylpyridine-2,6(1H,3H)-dione (**10**).—The above (10.5 g) cooled in ice and under nitrogen was treated dropwise with an aqueous solution of methylamine (8.5 ml, 25—30% w/v) with stirring, which was continued while the temperature of the mixture was allowed to rise to room temperature during 2 h when the mixture became homogeneous. It was then kept overnight in a refrigerator and evaporated. The residue was boiled under reflux for 1.25 h with a solution of sodium (1.35 g) in methanol (65 ml) and then evaporated; the residue was then dissolved in water and extracted with ether (× 3). Acetic acid (9.5 ml) was added to the aqueous solution which was then extracted with dichloromethane. The extract was dried and evaporated and the residue was chromatographed with dichloromethane as eluant. The eluate was recrystallised from dichloromethane–ether to yield colourless crystals, m.p. 114—115 °C (5 g) (Found: C, 54.2; H, 5.7; N, 9.0%; M^+, 155.0587. $C_7H_9NO_3$ requires C, 54.2; H, 5.85; N, 9.0%; M^+, 155.0582); $\nu_{max.}$ 1 720, 1 665, and 825 cm^{-1}; δ 3.2 (3 H, s, NMe), 3.4 (2 H, s, CH_2), 4.2 (3 H, s, OMe), and 5.4 (1 H, s, CH).

5-Hydroxy-4-methoxy-1-methylpyridine-2,6(1H,3H)-dione (**2**).—A solution of the above (4.6 g) in water (106 ml) con-

taining sodium hydroxide (5.9 g) under nitrogen was stirred at 0 °C while potassium persulphate (9.8 g) was added. The mixture was kept in a refrigerator for 2 days and then cooled in ice while concentrated sulphuric acid (8.1 ml) was added with stirring under nitrogen. The mixture was heated at 100 °C for 1 h under nitrogen, cooled in ice, and treated with sodium sulphite (31.5 g) followed by a solution of sodium dithionite (11 g) in water (25 ml). The pH of the solution was then approximately 7.0. The mixture was then extracted exhaustively with chloroform in a continuous extractor (several hours). Evaporation of the chloroform extract gave a residue (1.9 g) which was chromatographed rapidly on silica (20 g) using chloroform as eluant. The combined eluates from the earlier, crystalline fractions (total 1.06 g) were dissolved in hot chloroform (10 ml) and the resulting solution was diluted with ether (25 ml), kept in a refrigerator, and later diluted with further ether (2 × 5 ml). The resulting crystals were collected, washed with ether, and dried to yield synthetic (**2**) (0.624 g). The filtrate was concentrated and diluted further with ether, yielding additional (**2**) (0.16 g). The combined yield represents 15.7% although somewhat higher yields were obtained when the experiment was carried out on a smaller scale. For analysis a sample was purified by sublimation at 110 °C/0.01 mmHg, followed by recrystallisation from chloroform–ether. The product was identical in all respects with a sample of **A** isolated from *Mercurialis perennis* (Found: C, 49.1; H, 5.2; N, 8.2%).

Acetylation of **A**.—(a) Acetic anhydride (0.7 ml) was added to trifluoroacetic acid (2 ml) cooled in ice and after 5 min compound **A** (0.1 g) was added. The solution was kept for 24 h at room temperature and then evaporated. The residue was dissolved in dichloromethane, and the solution was diluted with ether and then decanted from a small amount of purple material. When the solution was diluted further with ether, crystals (65 mg) separated and these were chromatographed. The residue from the chloroform eluate was recrystallised from dichloromethane to afford colourless needles (51 mg), m.p. 150—151 °C (Found: C, 51.5; H, 5.0; N, 5.5%; M^+, 255.0751. $C_{11}H_{13}NO_6$ requires C, 51.75; H, 5.15; N, 5.5%; M^+, 255.0743); $\nu_{max.}$ 1 785, 1 755, 1 675, 1 620, 1 165, 865, and 785 cm^{-1}; δ 2.35 (3 H, s, OCOMe), 2.4 (3 H, s, OAc), 3.4 (3 H, s, NMe), 4.2 (3 H, s, OMe), and 5.95 (1 H, s, CH), (**13**) or (**14**).

(b) Compound **A** (40 mg) was added to a mixture of dry pyridine (1 ml) and acetic anhydride (0.25 ml) under nitrogen at room temperature when it dissolved without colouration. The solution was kept for 2 days at room temperature under nitrogen and then evaporated. The residue was dissolved in a little cold chloroform and the solution was diluted with ether. A small amount of purple solid was removed and the clear solution evaporated at room temperature. The residue was chromatographed and the chloroform eluate was recrystallised from chloroform–ether to afford needles of an acetoxyacetylhydroxy-4-methoxy-1-methylpyridin-2(1*H*)-one; *m/z* 255, 213, 198, 195, 171, and 157; M^+ ($C_{11}H_{13}NO_6$) 255; $\nu_{max.}$ 1 770, 1 670, 1 615, 1 205, 900, and 780 cm^{-1}; δ 2.2 (3 H, s), 2.5 (3 H, s), 3.35 (3 H, s), and 4.40 (3 H, s); δ(CF_3CO_2H) 2.5 (3 H, s), 2.7 (3 H, s), 3.6 (3 H, s), and 4.3 (3 H, s).

Action of Diazomethane on **A**.—An excess of ethereal diazomethane solution was added to an ice-cold solution of **A** (0.158 g) in methanol (3.5 ml) under nitrogen, and the mixture was kept in a refrigerator for 3 days. The solvent was removed and the residue was chromatographed on silica (5 g).

(a) Dichloromethane eluted a white solid (42 mg), which when recrystallised from chloroform–ether afforded (**15**) (21 mg) (Found: C, 52.75; H, 5.05; N, 7.6. $C_8H_9NO_4$ requires C, 52.45; H, 4.95; N, 7.65%); $\nu_{max.}$ 1 715, 1 675, 1 635, and 910 cm^{-1}; *m/z* 183, *182*, 167, 153, and 112; M^+ ($C_8H_9NO_4$) 183; δ 3.3 (3 H, s, NMe), 3.45 (2 H, CH_2), 3.8 (3 H, s, OMe), and 5.65 (1 H, s, CH).

(b) Chloroform eluted pale yellow crystals (44 mg) which when recrystallised from chloroform–ether gave cream crystals (12 mg) (Found: C, 51.8; H, 5.9; N, 7.4. Calc. for $C_8H_{11}NO_4$: C, 51.9; H, 6.0; N, 7.5%); *m/z* 185, 142, 128, and 100; $\nu_{max.}$ 1 705, 1 665, and 1 625; δ 1.6, 3.2, 3.8, and 5.3 (all s).

Action of Phenylhydrazine on **A**.—Compound **A** (41.5 mg) was added to a solution of phenylhydrazine hydrochloride (90 mg) and crystalline sodium acetate (140 mg) in water. The mixture was warmed for 1 min in a water-bath and then kept overnight at room temperature. The resulting precipitate was collected, washed with water, and dried. The product (45 mg) when recrystallised from methanol afforded dark red crystals (5 mg) readily soluble in chloroform; M^+ 351; $C_{19}H_{21}N_5O_2$ requires 351.

$\lambda_{max.}$(methanol)	260	330	440 nm
Dehydroascorbic acid osazone[19] $\lambda_{max.}$	266	348	441
Cyclohexane-1,2-dione osazone $\lambda_{max.}$	259	306	386

Oxidation Products of **A**.—(*a*) 4-*Methoxy*-1-*methylpyridine*-2,3,6-*trione* (**16**).—(i) Concentrated nitric acid (0.15 ml) was added to **A** (0.15 g) at −40 °C and the mixture allowed to gradually come to room temperature; it was then diluted with water and extracted with dichloromethane. The dried extract was evaporated and the residue was chromatographed rapidly. The product eluted by dichloromethane was recrystallised from chloroform—ether and then sublimed at 125 °C/0.01 mmHg, to afford (**16**) as pale yellow crystals, m.p. 132 °C (Found: C, 49.55; H, 4.0; N, 8.2. $C_7H_7NO_4$ requires C, 49.7; H, 4.15; N, 8.3%); $\nu_{max.}$ 1 730sh, 1 710sh, 1 670, 1 625, 1 310, 1 170, 1 110, 950, and 875 cm^{-1}; M^+, 169, small 171 (M + 2); $C_7H_7NO_4$ requires M^+, 169; δ 3.35 (3 H, s, NMe), 3.9 (3 H, s, OMe), and 6.25 (1 H, s, CH); δ(CF_3CO_2H) 3.45 (3 H, s, NMe), 4.0 (3 H, s, OMe), and 6.5 (1 H, s, CH). The same product was obtained by similar treatment of **B**.

(ii) Compound **A** (17 mg) was dissolved in phosphate buffer, pH 7.2 (20 ml).[20] A solution of $K_3[Fe(CN)_6]$ (0.658 g per 25 ml in the same buffer) was rapidly added dropwise, the solution becoming blue, then yellow when 2 ml (*i.e.* 2 equiv.) had been added. The solution was immediately extracted with chloroform and the dried extract on evaporation yielded (**16**).

(iii) A solution of 1,4-benzoquinone (20 mg) in ether was added to one of **A** (20 mg) in water (3 ml) under nitrogen. The mixture was shaken for a few seconds, extracted with ether, and the aqueous layer was then extracted with chloroform. Evaporation of the dried chloroform extract afforded (**16**) (10 mg).

(*b*) 5,5′-*Dihydroxy*-4,4′-*dimethoxy*-1,1′-*dimethyl*-3,3′-*bipyridine*-2,2′,6,6′(1H,1′H,3H,3′H)-*tetraone* (**18**). A solution of **A** (0.614 g) in methanol (4 ml) was boiled under reflux for 10 min and kept at room temperature for 5 h; it was then diluted with water and kept in a refrigerator. The product (0.49 g) was filtered off, washed with ether, dried, and recrystallised from acetic acid (4 ml). The colourless crystals were filtered off, washed with ether, and dried at 60 °C/0.5 mmHg, to afford compound **B** (**18**) (0.43 g) (Found: C, 49.2; H, 4.65; N, 8.2. $C_{14}H_{16}N_2O_8$ requires C, 49.41; H, 4.75; N, 8.25%); $\nu_{max.}$ 3 400, 1 725—1 635, 1 200, 900, 835, 815, and 760 cm^{-1}; $\lambda_{max.}$ 266 nm; δ[$(CD_3)_2SO$] 3.2 (3 H, t?, NMe), 3.8 (3 H, t?, OMe), 4.3 (1 H, d?, CH), and 8.5 (1 H, br, OH); δ(CF_3CO_2H) 3.45, 3.9, 4.0, 4.1, 4.3, and 6.5 (all s).

Acetic anhydride (0.5 ml) was added to ice-cold trifluoroacetic acid (1.5 ml). After 5 min, this mixture was added to compound **B** (74 mg) at 0 °C and the mixture was allowed to slowly come to room temperature. After 40 h the solution was evaporated and the residue chromatographed on silica (3 g).

The small amount eluted by dichloromethane was discarded. The subsequent chloroform eluate was recrystallised from chloroform–ether; yield 61 mg. After further recrystallisation from dichloromethane–ether the *acetyl* derivative (**19**) or (**20**), m.p. 174—175 °C (decomp., with gas evolution) was obtained (Found: C, 51.8; H, 4.75; N, 5.40%; M^+, 508.1313. $C_{22}H_{24}N_2O_{12}$ requires C, 51.95; H, 4.75; N, 5.51; M^+, 508.1329); $\nu_{max.}$ 1 780, 1 655, and 1 620 cm^{-1}; δ 2.15 (6 H, s, 2 OAc), 2.3 (6 H, s, 2 OAc), 3.35 (6 H, s, 2 NMe), and 3.8 (6 H, s, 2 OMe).

A mixture of **B** (0.2 g), 2,2′-azo-2-methylpropionitrile (0.25 g), and acetic acid (3 ml) was boiled under reflux for 4 h under nitrogen, then evaporated. The residue was chromatographed, using chloroform as eluant. The residues left by evaporation of the early eluates were soluble in ether, but subsequent fractions when treated with ether yielded crystals of 3-(2-*cyanopropan*-2-*yl*)-5-*hydroxy*-4-*methoxyl*-1-*methylpyridine*-2,6(1H,3H)-*dione* (**21**). This product was sublimed at 145 °C/0.1 mmHg and then recrystallised from methanol–ether to afford colourless needles, m.p. 152 °C (Found: C, 55.35; H, 5.55; N, 11.5%; M^+, 238.0953, base peak, 170. $C_{11}H_{14}N_2O_4$ requires C, 55.45; H, 5.9; N, 11.75%; M^+, 238.0953); $\nu_{max.}$ 2 250, 1 710, 1 670, and 1 630 cm^{-1}; δ 1.3 (3 H, s, CMe), 1.4 (3 H, s, CMe), 2.5 (3 H, s, NMe), 3.8 (3 H, s, OMe), 4.0 (1 H, s, CH), and 5.2 (1 H, s, OH).

A solution of **B** in chloroform was stirred overnight at room temperature with an aqueous solution of sodium dithionite. Extraction with chloroform, drying the extract with sodium sulphate–sodium dithionite, evaporation, and chromatography of the residue afforded **A**.

(*c*) *The Yellow Compounds* 5,5′-*Dihydroxy*-4,4′-*dimethoxy*-1,1′-*dimethyl*-3,3′-*bipyridylidene*-2,2′,6,6′-*tetraone* (**22**) *and* 4,4′-*Dimethoxy*-1,1′-*dimethyl*-3,3′-*bipyridine*-2,2′,5,5′,6,6′-*hexaone* (**23**).—(i) Compound **B** (0.1 g) was dissolved in pure dry pyridine (1 ml) under nitrogen. The solution was then exposed to air at room temperature. After 24 h the solution was evaporated and the residue was chromatographed. A yellow band was eluted by chloroform and when the eluate was evaporated it left a dark residue. The same product was obtained from **A** by similar treatment; δ 3.35 (3 H, s, NMe), 4.15 (3 H, s, OMe); *m/z* 336 > 338, *321*. This was heated with 0.2M HCl on a water-bath for 1 h and the product chromatographed. Elution with chloroform afforded a deep yellow solid (**23**) (Found: C, 49.7; H, 3.85; N, 8.2. $C_{14}H_{12}N_2O_8$ requires C, 50.0; H, 3.6; N, 8.3%); *m/z* 336 ≫ 338, *321*, no 169; $\nu_{max.}$ 3 420, 1 715, 1 660, 1 325, 1 110, and 735 cm^{-1}.

(ii) A solution of ceric ammonium nitrate (0.12 g) in a mixture of water (0.9 ml) and acetic acid (0.9 ml) was added to a mixture of **B** (30 mg) and acetic acid (0.9 ml) on a water-bath and the mixture was kept warm for 15 min; it was then cooled, diluted with water, and extracted with dichloromethane. The extract was washed with sodium hydrogen carbonate solution, dried, and evaporated. The residue was chromatographed. Dichloromethane eluted almost nothing, but chloroform eluted a yellow band. The material from the latter, when recrystallised twice from dichloromethane–ether, afforded a yellow product (13 mg) (Found: C, 49.85; H, 3.6; N, 8.1. $C_{14}H_{12}N_2O_8 \cdot C_{14}H_{14}N_2O_8$ requires C, 49.85; H, 3.85; N, 8.3%); *m/z* 338, 336, *321*, and 169; $\nu_{max.}$ 3 410, 1 715, 1 655, 1 320, 1 110, and 740 cm^{-1}; δ 3.35, 3.9, 4.15, and 6.25 (all s); δ(CF_3CO_2H) 3.45, 4.0, 4.3, and 6.5 (all s).

(iii) A similar experiment on **A** in which ceric ammonium nitrate (0.3 g) was added all at once yielded (**16**) as the main product.

(iv) An aqueous extract of *Mercurialis perennis* prepared as described above up to the point immediately before addition of sodium dithionite was shaken with air until the initially formed blue colour was replaced by brown. It was then filtered through HiFlo Supercel, extracted with chloroform, and the dried extract evaporated, and the residue chromatographed. The yellow material eluted by chloroform was recrystallised twice from chloroform–ether to afford an orange-yellow solid; M^+, 336.0604 (Calc. for $C_{14}H_{12}N_2O_8$: 336.0594) and M^+, 338.0746 (Calc. for $C_{14}H_{14}N_2O_8$: 338.0750); *m/z* 336 and 338 approx. equal; $\nu_{max.}$ 3 430, 1 715, 1 660, 1 320, 1 110, and 740 cm^{-1}; δ in $CDCl_3$ and CF_3CO_2H as above.

(v) A mixture of Sørensen's Na_2HPO_4 (18 ml) and KH_2PO_4 solution (12 ml)[20] was stirred in ice while carbon dioxide was passed through. Sodium borohydride (60 mg) was added, followed by **A** (22 mg). Further KH_2PO_4 solution (20 ml) was added and air, instead of carbon dioxide, was then passed through the solution. After 10 min the solution became deep blue, then green, and finally yellow. It was extracted with chloroform (× 3) and the extract was dried rapidly and evaporated, and the residue chromatographed. The yellow fraction (14 mg) eluted by chloroform was the same as the product obtained above from *Mercurialis perennis*.

Knackmuss' Compounds.—3,6-Dihydroxy-4-methylpyridin-2(1*H*)-one (**26**) (57 mg) was added to boiling water (5.7 ml) and the mixture heated for 2 h on a water-bath and then cooled. The crystals were filtered off, washed with water, methanol, and ether, and dried. The product (**27**) (43 mg) was added to concentrated nitric acid (0.07 ml) at −40 °C. The mixture was allowed to come to room temperature and diluted with water when the product (**28**) was filtered off, washed with water, methanol, and ether, and dried; it afforded a yellow solid (30 mg); M^+, 278.0550 ($C_{12}H_{10}N_2O_6$ requires 278.0539); *m/z* 276 negligible.

Potentiometric Titrations.—These were carried out using gold-plated platinum electrodes essentially as described by Cannan[5] except that they were done at room temperature. For pH 7.6 Sørensen's phosphate buffer,[20] and for pH 4 McIlvaine's buffer solution were used. 'Oxygen-free nitrogen' was purified by passage through Fieser's solution.

Synthesis of 3-*Amino*-6-*hydroxy*-4-*methoxy*-1-*methylpyridin*-2(1H)-*one* (**12**).—2M-HCl (7 ml) was added with stirring to a solution of (**10**) (1 g) in water (15 ml) cooled in ice. Sufficient of a solution of sodium nitrite (0.56 g) in water (2 ml) to produce a slight excess of nitrous acid was added dropwise and the mixture was then left in a refrigerator. The product (1.14 g) was filtered off, washed with water, and dried to yield 4-*methoxy*-1-*methyl*-3-*oximinopyridine*-2,6(1H,3H)-*dione* [**11**) as a cream solid (Found: C, 45.7; H, 4.25; N, 15.15. $C_7H_8N_2O_4$ requires C, 45.65; H, 4.35; N, 15.2%); *m/z* 184, *183*, 166, 154, 139, 126, and 110; $M^{\cdot+}$ $C_7H_8N_2O_4$ 184; δ 3.29 (3 H, s, NMe), 3.9 (3 H, s, OMe), and 5.72 (1 H, s, CH).

The above compound was stirred with concentrated hydrochloric acid (8 ml) with ice cooling while powdered tin (1.2 g) was gradually added. The pale yellow solid gradually dissolved, but later a precipitate separated. The mixture was allowed to come to room temperature, when most of the tin dissolved. The solution was evaporated and the residue dried; the latter was then dissolved in water and the resulting solution saturated with hydrogen sulphide. The filtered solution was evaporated to dryness and the residue dried and then dissolved in hot, concentrated hydrochloric acid (1.5 ml); the resulting solution was diluted with water (1.5 ml) and then kept in a refrigerator. Crystals (0.285 g) were filtered off and further crystals separated from the slightly diluted filtrate. These crystals were recrystallised from methanol–ether and consisted of 2,3,6-*trihydroxy*-4-*methoxy*-1-*methylpyridinium* (**1**) *chloride* (Found: C, 40.55; H, 4.7; N, 6.75. $C_7H_{10}ClNO_4$ requires C, 40.5; H, 4.8; N, 6.75%).

An experiment as above except that the crude residue left

after evaporation of the solution treated with hydrogen sulphide was sublimed at 155 °C/0.01 mmHg to yield a colourless solid, 3-*amino*-6-*hydroxy*-4-*methoxy*-1-*methylpyridin*-2(1H)-*one* (**12**); *m/z* 170, 155 ($C_7H_{10}N_2O_3$ requires 170).

Dimethyl Methoxymalonate.—This compound was prepared as described by Ames and Bowman[21] for the diethyl ester except that methyl methoxyacetate (43.5 g) was added dropwise during 2 h to a suspension of methanol-free sodium methoxide (from sodium, 10.6 g) in dimethyl carbonate (330 ml) with continuous distillation of methanol. A solution of the product (21.55 g; b.p. 85—95 °C/1 mmHg) in ether was shaken with aqueous sodium hydrogen sulphite (40%; 4 ml), dried, and redistilled to give the pure ester (18.3 g; b.p. 85 °C/1 mmHg); δ(CCl_4) 3.45 (3 H, s, OMe), 3.75 (6 H, s, 2 CO_2Me), and 4.25 (1 H, s, CH).

Methoxy(*methylaminomethyl*)*malonic Acid* (**33**).—A mixture of the above ester (2 g) and a methanolic solution of potassium hydroxide (1.4 g) was boiled under reflux for a few minutes and then cooled; the salt which separated was filtered off, washed with methanol followed by ether, and then dried. Concentration of the filtrate afforded a further crop of salt (total 2.35 g). The two crops of salt were combined and stirred with a cold mixture of concentrated sulphuric acid (0.62 ml) and water (2.2 ml). Part of the water was removed rapidly using a rotary evaporator and the residue was dried over phosphoric oxide in a desiccator. The residue was extracted with boiling acetone and the extract was evaporated to dryness. The residue from the acetone solution was then extracted with boiling ether. Evaporation of the ethereal extract yielded a gum which rapidly crystallised to afford methoxymalonic acid (1.28 g).

A solution of this (0.64 g) in water (0.48 ml) was neutralised by methylamine (25—30% w/v aqueous solution; *ca.* 0.94 ml). Further methoxymalonic acid (0.64 g) was then added, followed by aqueous formaldehyde (40%; 0.83 ml) and the solution was kept for 4 days at room temperature. The resulting crystals (0.7 g) were filtered off, washed with methanol, followed by ether, and then dried. The combined filtrates deposited further *acid* (0.02 g) (Found: C, 40.55; H, 6.2; N, 7.9. $C_6H_{11}NO_5$ requires C, 40.7; H, 6.25; N, 7.9%); *m/z* 134, 133, and 102 ($C_6H_{11}NO_5$ requires 177; $C_5H_{11}NO_3$ requires 133); δ(D_2O) 2.75 (3 H, s, NMe), 3.45 (3 H, s, OMe), and 3.55 (2 H, s, CH_2); $\nu_{max.}$ 3 430, 3 050, 1 725, 1 600, 850, and 750 cm^{-1}.

Methyl 2-*Methoxy*-3-*methylaminopropanoate* (**34**).—(a) The above acid (0.6 g) was heated in water (4 ml) on a water-bath until evolution of carbon dioxide ceased (*ca.* 15 min). Concentrated hydrochloric acid (0.4 ml) was added to the resulting solution which was then evaporated to dryness. The residue was heated under reflux for 20 min with an excess of pure thionyl chloride and the solution was then evaporated to dryness. A solution of the dried residue in methanol was gradually diluted with ether until crystallisation occurred and the mixture was then kept overnight in a refrigerator. The resulting *hydrochloride* of (**34**) (0.43 g) was filtered off, washed with ether, and dried; δ(D_2O) 2.8 (3 H, s, NMe), 3.75 (3 H, s, OMe), 3.85 (3 H, s, CO_2Me), 3.4 (2 H, d, CH_2), and 4.4 (1 H, t, CH).

(b) A solution of potassium hydroxide (1.62 g) in methanol (14 ml) was added to one of dimethyl methoxymalonate (4.64 g) in methanol (14 ml) and the mixture was kept overnight at room temperature; it was then evaporated to dryness. To a solution of the residual gum in water (3.2 ml) was added methylammonium chloride (1.96 g), followed by aqueous formaldehyde (40%; 2.4 ml), and then acetic acid (1.2 ml). The mixture was kept for 2 days at room temperature and then evaporated to dryness. The residue was treated with methanol and the mixture again evaporated. Dry methanol was added to the residue and the mixture was saturated with hydrogen chloride at 0 °C; it was then kept overnight at room temperature, boiled under reflux for 2 h, and evaporated to dryness. A solution of the residue in methanol was diluted with ether to afford a hydrochloride (4.4 g) identical with the above.

When the Mannich reaction was carried out as above except that the amount of acetic acid was considerably increased, crystals separated when the solution was kept at room temperature, and recrystallisation of these from methanol afforded *methyl methoxy*(*methylaminomethyl*)*malonate* (**35**); *m/z* 147 and *116* ($C_7H_{13}NO_5$ requires 191; $C_6H_{13}NO_3$ requires 147); δ(D_2O) 2.8 (3 H, s, NMe), 3.5 (3 H, s, OMe), 3.6 (2 H, s, CH_2), and 3.85 (3 H, s, OMe).

An aqueous solution of the above hydrochloride was saturated with potassium carbonate and then extracted with ether; distillation of the dried (K_2CO_3) extract yielded the base (**34**), b.p. 55 °C/1 mmHg; *m/z* 147 and 116 ($C_6H_{13}NO_3$ requires 147); δ(CCl_4) 2.4 (3 H, s, NMe), 2.8 (2 H, d, CH_2), 3.4 (3 H, s, OMe), and 3.8 (OMe and NH).

5-*Methoxy*-1-*methylpiperidine*-2,4-*dione* (**38**).—A solution of sodium carbonate (9.5 g) in water (130 ml) was stirred with ether (170 ml) with ice-cooling. The hydrochloride of (**34**) (5.45 g) was added, followed during 25 min with a solution of ethoxycarbonylacetyl chloride (13.7 g) in ether (100 ml). The mixture was stirred for a further 1 h with ice-cooling after which the aqueous layer was separated and extracted exhaustively with ether. Evaporation of the combined and dried extracts afforded the amide (**36**) as a gum (7.12 g); *m/z* 261, 216, 202, *158*, 146, 137, 115, and 114 ($C_{11}H_{19}NO_6$ requires 261). A solution of this in xylene (20 ml) was added to powdered sodium (0.795 g) under xylene (20 ml) and the mixture was gradually brought to reflux temperature under nitrogen; it was then kept at this temperature for 1 h after which it was cooled and treated with water. The aqueous layer was cooled in ice and treated with a mixture of concentrated hydrochloric acid (4 ml) and water (4 ml); it was then extracted with chloroform. Evaporation of the dried extract yielded the oxo-ester (**37**) (5.337 g), which was boiled under reflux for 1.5 h under nitrogen with 10% acetic acid and then evaporated to dryness. The dried residue was chromatographed on Hopkin Williams silica (50 g). The earlier eluates were almost completely soluble in ether, and these were followed by very viscous gums sparingly soluble in ether. Then followed crystalline fractions of the dione (**38**) (1.38 g), purified by sublimation *in vacuo*; $M^{\cdot+}$ 157 ($C_7H_{11}NO_3$ requires M^+, 157); δ 3.05 (3 H, s, NMe), 3.4—3.6 (4 H, m, 2 CH_2), 3.5 (3 H, s, OMe), and 3.9 (1 H, t, C*H*OMe).

3-*Diazo*-5-*methoxy*-1-*methylpiperidine*-2,4-*dione* (**39**).—Amberlite XE-305 (3 g) was treated with a solution of chlorosulphonic acid (3 ml) in pure chloroform (30 ml) for 30 min at room temperature after which it was boiled under reflux for 30 min. The mixture was then cooled and filtered, and the resin was washed with carbon tetrachloride, boiled under reflux for 30 min with carbon tetrachloride, cooled, filtered, washed with carbon tetrachloride, and dried. The resin was then stirred with water, filtered off, washed with water, and then stirred overnight with a solution of sodium azide (6 g) in a mixture of water (30 ml), dioxane (30 ml) and ethanol (30 ml). It was then filtered off, washed successively with the latter solvent mixture, water, and ethanol, and then dried; yield 5.35 g. The product was stored *in vacuo* over phosphoric oxide.

The above resin (1.55 g) was stirred at 0 °C with a solution of the dione (**38**) (0.129 g) in dry methanol (13 ml) while triethylamine (0.45 ml) was added; the mixture was then stirred for 2.8 h (with protection from moisture) after which acetic acid (0.68 ml) was added. The mixture was centrifuged and the resin washed with methanol. The solution was evaporated and a

solution of the residue in dichloromethane was shaken with very dilute acetic acid, dried, and evaporated. Chromatography of the residue yielded the diazoketone (**39**) (0.055 g); *m/z* 183 and 155 ($C_7H_9N_3O_3$ requires 183; $C_7H_9NO_3$ requires 155).

3,4-*Dihydroxy*-5-*methoxy*-1-*methylpyridin*-2(1H)-*one* (**7**).—t-Butyl hypochlorite (0.11 ml) was added to a solution of the diazoketone (**39**) (0.1 g) in formic acid (0.4 ml) at 0 °C. After 30 min at room temperature the solution was evaporated to dryness. A solution of the residue (0.12 g) in dichloromethane was gradually diluted with ether at 0 °C and the resulting white solid was collected and sublimed twice at 150 °C/0.01 mmHg. The sublimate was recrystallised from dichloromethane–methanol and the crystals were extracted with dichloromethane. The insoluble part was dissolved in hot methanol and the solution diluted with ether and then kept overnight in a refrigerator. The product was filtered off, washed with ether, and dried to afford the *pyridinone* (**7**) as colourless needles (14.5 mg); *m/z* 171, 156, 153, 141, and 100 (M^+, 171.0530. $C_7H_9NO_4$ requires *M*, 171.0532); δ(CF_3CO_2H) 4.0 (6 H, NMe and OMe) and 7.2 (1 H, CH); $\nu_{max.}$ 3 300, 1 665w, 1 535, 1 495, 920, 805, and 760 cm^{-1}.

When the above ketone was heated for 2 h on a water-bath with acetic anhydride it afforded a *diacetyl* derivative, *m/z* 255, 213, 171, 153, 141, 125, and 100; M^+, 255.0760; $C_{11}H_{13}NO_6$ requires 255.0743; δ 2.31 (3 H, s, OAc), 2.33 (3 H, s, OAc), 3.56 (3 H, s, NMe), 3.72 (3 H, s, OMe), and 6.75 (1 H, s, CH); $\nu_{max.}$ 1 770, 1 670, and 1 620 cm^{-1}.

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