contain a compound identified by ionophoresis as γ -hydroxyhomoarginine.

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APPENDIX

Infrared-Absorption and Nuclear-Magnetic-Resonance Spectra of the Lactones of γ -Hydroxyarginine and γ -Hydroxyhomoarginine

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The availability of authentic γ -hydroxyarginine and γ -hydroxyornithine made direct confirmation of the identity of the guanidino amino acid isolated, as its lactone, from seeds of *Vicia sativa* and the product of its alkaline hydrolysis possible. The guanidino amino acid found in the seeds of *Lathyrus tingitanus*, however, had not previously been described, and, although on the reported evidence its identity as γ -hydroxyhomoarginine, and that of the isolated artifact as the corresponding lactone, appeared unambiguous, it was desirable to obtain confirmation by independent methods.

Infrared-absorption spectra. Spectra were determined with a double-beam spectrophotometer by using the KI-pressed-disk technique and a rock-salt prism. The spectrum of the artifact from *L. tingitanus* showed an absorption band at 5.68μ , as did that from *V. sativa*. Absorption in this region of the spectrum is characteristic of γ -lactones (Bellamy, 1958), reconfirming the identity of γ -hydroxyarginine in *V. sativa* and confirming that of γ -hydroxyhomoarginine in *L. tingitanus*.

When the artifact from *L. tingitanus* was dissolved in N-ammonia and the solution evaporated to dryness (*in vacuo* at room temperature) the residue showed no absorption at this frequency. When the disk prepared from this residue was exposed to an atmosphere containing hydrochloric acid for 17 hr. and then heated at 150° *in vacuo* for a further 1 hr. it was found that the band at 5.68μ reappeared though at reduced intensity. These findings are consistent with the opening of a γ -lactone ring in alkaline solution and its partial re-formation.

Nuclear-magnetic-resonance spectra. On the basis of the chemical evidence and infrared-absorption measurements described, the structures of γ -hydroxyarginine lactone (I) and γ -hydroxyhomoarginine lactone (II) can be assigned to the artifacts isolated from the seeds of *V. sativa* and *L. tingitanus* respectively.

The nuclear-magnetic-resonance spectra of these two compounds (measured as solutions in D_2O at 56.4 Mcyc./sec.) are in accordance with these assignments.

Table 1. *Nuclear-magnetic-resonance spectra of hydrogen atoms in the isolated lactones*

The chemical shifts are expressed in p.p.m. from internal 2-methylpropan-2-ol.

Position of protons	Chemical shift (p.p.m.)	
	Lactone (<i>V. sativa</i>)	Lactone (<i>L. tingitani</i>)
3	1.42	1.75
5	2.36	0.88
6	—	2.18

The spectrum of the compound from *V. sativa* shows two bands, the more complex one, at higher field, is the AB portion of an ABX spectrum which has been further split by a single proton. The lower field band is the AB part of an ABX spectrum. The position and multiplicities of these bands in the observed spectrum are those expected of the 3- and 5-protons of the proposed formula. The 2- and 4-proton signals are masked by the band arising from the few per cent of water in the commercial D₂O used as solvent.

The most prominent feature of the spectrum of the compound from *L. tingitani* is the triplet at a shift of 2.18 p.p.m. This has been assigned to the 6-protons in formula (II): the triplet splitting, and the small upfield shift of this band compared with the corresponding band in the spectrum of the compound from *V. sativa*, is in agreement with the introduction of a —CH₂— grouping between the C-4 and C-5 of formula (I). Two other complex bands were observed in the spectrum of this compound and their positions and multiplicities are suitable for the 3- and 5-protons of formula (II). Again the methine protons are masked by the solvent line.

These spectra are in agreement with the chemical evidence and indicate that the compounds isolated from *V. sativa* and *L. tingitani* are the lactones derived respectively from γ -hydroxyarginine and γ -hydroxyhomoarginine.

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The Biochemistry of Aromatic Amines

10. ENZYMIC *N*-HYDROXYLATION OF ARYLAMINES AND CONVERSION OF ARYLHYDROXYLAMINES INTO *o*-AMINOPHENOLS*

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Since the finding that one of the routes of metabolism of 2-acetamidofluorene in the rat is by *N*-hydroxylation and subsequent conjugation with glucuronic acid (Cramer, Miller & Miller, 1960) it has been shown that 2-naphthylamine (Troll & Nelson, 1961), 4-acetamidobiphenyl (Miller, Wyatt, Miller & Hartmann, 1961*b*), 4-aminostilbene (Miller & Miller, 1962) and benzidine (Troll, Belman & Rinde, 1963) are also excreted in urine as *N*-hydroxy metabolites by a variety of species. This reaction is likely to play an important part in the carcinogenic properties of the arylamines since the *N*-hydroxy derivatives of 2-acetamidofluorene (Miller, Miller & Hartmann, 1961*a*), 4-acetamidobiphenyl (Miller *et al.* 1961*b*), 4-acetamidostilbene (Anderson, Enomoto, Miller & Miller, 1963) and

2-naphthylhydroxylamine (Boyland, Dukes & Grover, 1963) are more potent carcinogens than the parent compounds under certain experimental conditions.

The *N*-oxidation of several arylamines by rat-liver microsomes and NADPH has been demonstrated by the estimation of the hydroxylamine and nitroso derivatives (Uehleke, 1961, 1963; Kampffmeyer & Kiese, 1963). The formation of 2-(*N*-hydroxyacetamido)fluorene from 2-acetamidofluorene by rabbit-liver microsomes has been detected and estimated (Irving, 1962*a*).

The present paper describes the enzyme systems in rabbit liver which are concerned with the *N*-hydroxylation of acetamidoaryl compounds, with special reference to 4-acetamidobiphenyl, and the further reactions of the metabolic *N*-hydroxy-acetamidoaryl compounds.

* Part 9: Boyland, Manson & Nery (1963).