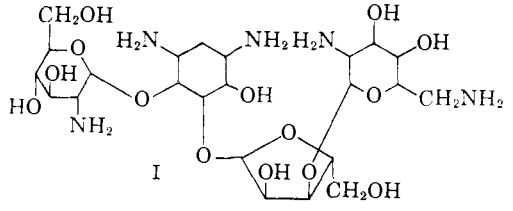
to adjacent hydroxyls in deoxystreptamine as shown in I.



I

Additional support for a 5,6(4,5) linkage also can be rationalized on the basis of the extreme ease of methanolysis of paromomycin (0.32 *N* HCl) in contrast to kanamycin which is unaffected under these conditions and has been shown to be a 4,6-disubstituted deoxystreptamine.[2] Final proof for structure I was obtained from methylation and subsequent hydrolysis experiments.

When N-pentacetylparomomycin was methylated by a modified procedure of West and Holden,[3] a product containing 22.2% methoxyl (26.4% for full methylation) was obtained. Acid hydrolysis followed by ion exchange and cellulose chromatography afforded an optically active mono-*O*-methyldeoxystreptamine isolated as the crystalline N,N'-diacetyl derivative. *Anal.* Found: C, 50.38; H, 7.76; N, 10.72; $[\alpha]^{27}D$ +15° (*c* 1.0, H_2O); m.p. 280–282° dec. The product consumed 1.0 mole of periodate in 24 hours at 5°. The isolation of an optically active *O*-monosubstituted deoxystreptamine conclusively places the methoxyl in either the 4 or 6 position since 5 substitution produces a *meso* form.[4]

From the neutral fraction of the above hydrolyzate there was isolated a sugar which corresponded to 2,5-di-*O*-methyl-D-ribose by paper chromatography and electrophoresis.[5] Final characterization was accomplished by conversion to the crystalline *p*-bromophenylosazone which melted at 183–184° and showed no depression on mixing with an authentic sample.[6] *Anal.* Found: C, 42.89; H, 4.14; N, 11.09. The isolation of this disubstituted ribose proves the presence of a furanose ring structure in the intact antibiotic and confirms the glycosidic attachment of paromose to the third carbon of D-ribose.

Since the molecular rotation of an unsymmetrically monosubstituted deoxystreptamine is now known (+3900) it is possible to calculate by Hudson's rules the anomeric contribution (A_G) of the glycosidic linkage in paromamine. Since 4 and 6 monosubstituted deoxystreptamines are enantiomorphs, the contribution of the deoxystreptamine moiety ($[M]_D$) in paromamine must be of opposite sign to the methoxy derivative. By appropriate substitution into the equation $[M]_p = [M]_D + A_G + B_G$ and solving for A_G, a value of +27,070 is obtained. This is in agreement with the calculated values (± 25,000 ± 5,000)[7,8] for the contribution of anomeric centers in alkyl glycosides. Since the sugar is in the D-series, the positive value establishes an α-D-linkage in paromamine.

(2) M. J. Cron, O. B. Fardig, D. L. Johnson, D. F. Whitehead, I. R. Hooper and R. U. Lemieux, THIS JOURNAL, **80**, 4115 (1958).

(3) E. S. West and R. F. Holden, *ibid.*, **56**, 930 (1934).

(4) S. Umezawa, Y. Ito and S. Fukatsu, *J. Antibiotics (Japan)*, **A11**, 162 (1958).

(5) D. M. Brown, D. I. Magrath and A. R. Todd, *J. Chem. Soc.*, 1442 (1954).

(6) The authors are indebted to Professor A. R. Todd and coworkers for this service.

(7) C. S. Hudson, THIS JOURNAL, **31**, 66 (1909).

(8) R. U. Lemieux, C. W. DeWalt and M. L. Wolfrom, *ibid.*, **69**, 1838 (1947).

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ENZYMATIC FORMATION OF L-GLUTAMIC ACID AND ACETIC ACID FROM KYNURENIC ACID[1]

Sir:

Kynurenic acid (KA) has been shown to be degraded by resting cell suspensions and crude extracts of *Pseudomonas*,[2,3] but precise metabolic pathways have not yet been elucidated. We now are reporting the identification of L-glutamate and acetate as products of the degradation of KA by a partially purified enzyme preparation from a tryptophan adapted *Pseudomonas* sp. (ATCC 11299B).

Cells were grown and extracts were made as previously described.[4] Crude extracts then were centrifuged at 100,000 X *g* for one hour. The incubation mixture contained, in a final volume of 55.5 ml., 52 ml. of the supernatant fraction (468 mg. protein), 0.40 μM. of KA-3-C^{14} [5] (758,000 c.p.m.) and 304.0 μM. of unlabeled KA. The reaction mixture was incubated at 36° for 2.5 hours with continuous reciprocal shaking, deproteinized with cold 3 % H_2SO_4 and was extracted with 3 vol. of ether. The ether soluble fraction, which contained approximately 40% of the original counts, was subjected to partition chromatography on a Celite column.[6] A radioactive compound was identified tentatively as acetic acid by its titration curve, an enzymatic assay using acetokinase of *E. coli*[7] and by the melting point of *p*-bromophenacyl ester (85°).[8]

The water layer was neutralized with $Ba(OH)_2$ and centrifuged. The supernatant was subjected to ion exchange chromatography on Dowex-1 and Dowex-50 columns and L-glutamic acid hydro-

(1) This investigation was supported in part by research grants from the National Institutes of Health (C-4222), the Rockefeller Foundation and the Jane Coffin Childs Memorial Fund for Medical Research.

(2) R. Y. Stanier and O. Hayaishi, *Science*, **114**, 326 (1951).

(3) E. J. Behrman and T. Tanaka, *Fed. Proc.*, **18**, 189 (1959).

(4) O. Hayaishi, *Biochem. Preparations*, **3**, 108 (1953).

(5) DL-Tryptophan-3-C^{14} was converted enzymatically to kynurenine-3-C^{14},[4] which was further converted to KA-3-C^{14} by Pseudomonas transaminase in the presence of α-ketoglutarate (I. L. Miller, M. Tsuchida and E. A. Adelberg, *J. Biol. Chem.*, **203**, 205 (1953). KA was then purified by ion exchange chromatography and was shown to be chromatographically pure on paper using three different solvent systems. Details of this procedure will be published elsewhere.

(6) M. H. Peterson and M. J. Johnson, *ibid.*, **174**, 775 (1948).

(7) I. A. Rose, M. Grunberg-Manago, S. P. Korey and S. Ochoa, *ibid.*, **211**, 737 (1954).

(8) W. L. Judefind and E. E. Reid, THIS JOURNAL, **42**, 1043 (1920).

chloride (82 μM.) was isolated and crystallized as described before.[9] *Melting point:* 180–184° (authentic sample of L-glutamic acid HCl 184–188°; mixed m.p. 182–186°). *Anal.* Calcd. for $C_5H_9O_4N$ HCl: C, 32.71; H, 5.49; N, 7.63. Found: C, 32.91; H, 5.39; N, 7.67. $[\alpha]^{20}D = +31.7°$. Specific activity was calculated to be 1430 c.p.m. and 1560 c.p.m. on the basis of ninhydrin and enzymatic assays (see below), respectively (original specific activity of KA, 2460 c.p.m.). Further evidence for the identity was provided by paper chromatography[10] after and before enzymatic decarboxylation by L-glutamic decarboxylase.[11]

Preliminary experiments with KA-2-C^{14}, carboxyl-labeled KA and KA-9-C^{14} [12] indicated that glutamic acid is probably derived from the carboxyl carbon and carbon 2, 3, 4 and 10. Further studies are now in progress in order to identify other products and intermediate steps.

(9) H. Tabor and O. Hayaishi, *J. Biol. Chem.*, **194**, 171 (1952).

(10) Paper chromatographic analysis was carried out with three different solvent systems and examined with an automatic scanner and ninhydrin spray. The isolated material before and after enzymatic decarboxylation gave exactly the same R_f values as those of authentic samples of L-glutamic acid and γ-aminobutyric acid, respectively. R_f values for glutamic acid and γ-aminobutyric acid were as follows: 0.28 and 0.63 (butanol–acetic acid–water, 4:1:1), 0.15 and 0.56 (water saturated phenol), 0.35 and 0.52 (ethanol–ammonia–water, 18:1:1), respectively. DL-γ-Hydroxyglutamic acid, kindly furnished from Dr. T. Kaneko, gave R_f values of 0.14, 0.07 and 0.29, respectively.

(11) O. Schales, V. Mims and S. S. Schales, *Arch. Biochem.*, **10**, 455 (1946).

(12) KA-2-C^{14} and KA-9-C^{14} were synthesized from DL-tryptophan-2-C^{14} and DL-tryptophan-7α-C^{14}, respectively, as described above. The latter compound was a generous gift of Dr. M. Rothstein of University of California. Carboxyl-labeled KA was kindly furnished by Dr. J. M. Price of the University of Wisconsin.

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α-METALLOCENYL CARBONIUM IONS[1,2]

Sir:

The results of the solvolyses reported in Table I demonstrate that α-metallocenyl carbonium ions are remarkably stable. Indeed, the rates indicate that these α-metallocenyl cations are of the same order of stability as the triphenylmethyl cation. Qualitative observations such as the solubility of ferrocenecarboxaldehyde in dilute hydrochloric acid[3] and the facile conversion of phenylferrocenylcarbinol to the corresponding methyl ether by refluxing aqueous methanol[4] previously have implied a high order of stability for such ions.

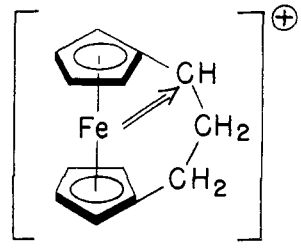
That these solvolyses indeed proceeded by alkyl-oxygen fission was conclusively shown by the ethanolysis of III and VI. Each of these acetates produced the corresponding ethyl ether and one mole of acetic acid. The addition of 7.16×10^{-3} M acetate ion reduced the solvolysis rate of III by a factor of nearly five. This was not caused simply by a change in the ionic strength of the medium as the addition of 7.24×10^{-3} M lithium perchlorate had a negligible effect on the solvolysis rate. The common ion rate depression is, therefore, further evidence for alkyl oxygen fission.[5] More importantly the large magnitude of the effect in an 80% acetone–water mixture indicates the intermediacy of an extremely stable ion which is quite selective in its recombination with nucleophiles.[6]

TABLE I[a,b]

		Rel. rate 30° in 80% acetone/water
I	Trityl acetate	1
II	Ferrocenylcarbinyl acetate[c]	0.63
III	Methylferrocenylcarbinyl acetate[d]	6.7
IV	Methylruthenocenylcarbinyl acetate[e]	9.0
V	Methylosmocenylcarbinyl acetate[e]	34
VI	α-Acetoxy-1,1′-trimethyleneferrocene[f]	0.23

[a] The synthesis of all compounds was accomplished by standard methods from known intermediates.[c,d,e,f] Satisfactory analyses were obtained for all acetates. [b] Rates were determined by aliquot titration of the acetic acid produced. The rates followed the first order law faithfully to at least 85% completion. [c] F. S. Arimoto and A. C. Haven, Jr., THIS JOURNAL, **77**, 6295 (1955). [d] P. J. Graham, R. V. Lindsey, G. W. Parshall, M. L. Peterson and G. M. Whitman, *ibid.*, **79**, 3416 (1957). [e] Ref. 7. [f] K. L. Rinehart, Jr., and R. J. Curby, Jr., *ibid.*, **79**, 3290 (1957).

The requirement for coplanarity of the cationic center and the cyclopentadienyl ring is demonstrated by the markedly slower solvolysis rate of the bridged acetate VI (slower by a factor of 132 than the corresponding secondary acetate III). It is to be emphasized, however, that the reaction proceeded by a carbonium ion mechanism as was indicated by the occurrence of alkyl-oxygen fission. This leads us to propose the possibility that this bridged, non-planar ion is stabilized by a direct participation of the iron electrons.



Another interesting feature of the relative rates recorded in Table I is the order of effectiveness of metallocene derivatives of Group VIII metals in stabilizing adjacent carbonium ion centers; ferrocene < ruthenocene < osmocene. This is just the inverse of the order of reactivity of these sub-

(1) Presented in part at the 135th Meeting of the American Chemical Society, Boston, Mass., April 5–10, 1959, *cf.* Abstracts p. 86-O.

(2) This work was supported in part by the National Science Foundation.

(3) G. D. Broadhead, J. M. Osgerby and P. L. Pauson, *J. Chem. Soc.*, 650 (1958).

(4) N. Weliky and E. S. Gould, THIS JOURNAL, **79**, 2742 (1957).

(5) For a general discussion, *cf.*, C. K. Ingold, "Structure and Mechanism in Organic Chemistry," Cornell University Press, Ithaca, N. Y., 1953, p. 360.

(6) C. G. Swain, C. B. Scott and K. H. Lohman, THIS JOURNAL, **75**, 136 (1953).