THE BIOGENESIS OF RICININE[1]

P.F. Juby[2] and Léo Marion

Division of Pure Chemistry, National Research Council,
Ottawa, Canada

Received July 28, 1961

It has been postulated that in some bacteria and some higher plants the pyridine ring of such compounds as nicotinic acid (Ortega and Brown, 1960), nicotine (Griffith, Hellman, and Byerrum, 1960), and the α-pyridone ring of ricinine (Waller and Henderson, 1960) may be synthesized from simple metabolic intermediates related to acetate, succinate, glycerol, or propionate. On the other hand, Tamir and Ginsberg (1959) claimed the specific incorporation of DL-lysine-2-C^{14} into ricinine.

The present study confirms the incorporation of several simple precursors into ricinine (Fig. 1) by Ricinus communis L. It is suggested that the results obtained, as well as the results of other workers, can best be explained by adopting the idea that succinic acid or a closely related 4-carbon dicarboxylic acid found in the Krebs tricarboxylic acid cycle is a direct precursor of ricinine.

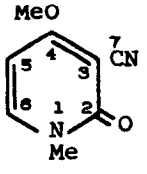

Fig. 1

EXPERIMENTAL

Seeds of Ricinus communis L. were planted in moist sand contained in glass trays placed in a dark cabinet at 20-25°. After 12 days the trays were brought out into the light for

---

[1] N.R.C. No. 6435.

[2] National Research Council of Canada Postdoctorate Fellow.

about 16 hours each day and the sand was kept moist with inorganic nutrient solution. On or around the 17th day of growth aqueous solutions (ca. 0.02 c.c.) of the radioactive precursors were introduced into each plant by means of a bent glass capillary tube inserted into the base of the plant stem. Uptake of the solution was usually complete within a few minutes.

The plants were harvested 72 hours after feeding and the wet stems and leaves were extracted with absolute ethanol. After removal of the ethanol from this extract the residue was suspended in water, washed with petroleum ether and then continuously extracted with chloroform. This last extract yielded crystalline ricinine which was purified by crystallization and sublimation, m.p. 202°.

For the determination of their radioactivities the methyl groups attached to oxygen and to nitrogen were removed by the Herzig-Meyer method (Pregl, 1945) and isolated as tetramethyl ammonium iodide which was recrystallized to constant activity.

To determine the radioactivity of the nitrile group ricinine was converted into ricinic acid and the latter oxidized with chromic acid (Böttcher, 1918). The liberated HCN was precipitated as AgCN.

In addition, the ricinine isolated from plants fed succinic acid-2,3-C^{14} was converted into 4-methoxy-1-methyl-2-pyridone (Winterstein et al., 1917), colorless crystals by sublimation, m.p. 114-116°. In this case the activity of the nitrile group was obtained by difference.

RESULTS AND DISCUSSION

The results obtained are listed in Tables 1 and 2.

Although the percentage incorporation into ricinine of DL-lysine-2-C^{14} monohydrochloride is of the same order as that obtained by Tamir and Ginsberg (1959), the incorporation is very low compared to that of the other precursors, and the radioactivity is obviously randomly distributed as shown by the relatively high percentage activities of the CN, OMe and NMe groups.

TABLE 1

Precursor	Wt. in mg.	Available c.p.m.	No. of plants fed	Ricinine		% Incorp. of precursor
				Yield (mg.)	Specific act. (cpm/mg)	
Sodium acetate-1-C^{14}	0.92	6.21×10^7	28	246	8440	3.3
Sodium acetate-2-C^{14}	6.05	1.45×10^8	42	353	2600	6.6
Succinic acid-2,3-C^{14}	0.85	3.64×10^7	56	415	3200	3.6
Glycerol-1-C^{14}	1.53	8.88×10^7	42	418	8203	3.9
Glycerol-2-C^{14}	2.13	8.88×10^7	50	470	5140	2.7
DL-Lysine-2-C^{14} monohydrochloride	29.4	7.19×10^7	55	351	69	0.05

TABLE 2

Precursor	% of activity contained in CN group	% of activity contained in the OMe and NMe groups combined
Sodium acetate-1-C^{14}	93.0	5.9
Sodium acetate-2-C^{14}	20.8	1.3
Succinic acid-2,3-C^{14}	19.6*, 27.0†	1.1
Glycerol-1-C^{14}	9.4	35.0
Glycerol-2-C^{14}	19.7	26.2
DL-Lysine-2-C^{14} monohydrochloride	29.2	28.0

* CN determined as AgCN
† CN determined indirectly

The most striking result is the high percentage activity of the nitrile group in ricinine obtained from sodium acetate-

1-C^{14}. Anwar et al. (1961) also report that degradations showed that 90% of the C^{14} from acetate-1-C^{14}, glutamate-2-C^{14}, propionate-1-C^{14} and propionate-3-C^{14} was located in the carbon of the nitrile group of ricinine. We feel that these results and most others can best be explained by simply adopting the idea of Waller and Henderson (1960) that succinic acid or a closely related 4-carbon dicarboxylic acid found in the tricarboxylic acid cycle is a direct precursor of ricinine. Beevers (1957) and Kornberg and Beevers (1957) have demonstrated the operation of the Krebs cycle and glyoxalate bypass in the endosperm and cotyledons of Ricinus seedlings. The 4-carbon unit would be incorporated in such a way that one of the carboxyl groups provides the carbon for the nitrile group of ricinine and the 2 and 3 carbons give rise to carbon in the 3 and 2 positions respectively of the pyridone ring. The other carboxyl group must eventually be lost by decarboxylation. The distribution of activity in ricinine shown for acetate and glutamate can now be accounted for by the operation of the Krebs cycle, with or without the glyoxalate bypass.

Feeding of succinic acid-2,3-C^{14} would result in succinate labelled on one carboxyl group after only one operation of the cycle. This type of randomization could account for the level of activity actually found on the nitrile group after feeding succinic acid-2,3-C^{14}. Acetate-2-C^{14} would produce succinate labelled predominantly on the methylene groups but with some carboxyl labelling.[3] Acetate-1-C^{14} could only form carboxyl labelled succinate and this would account for the high proportion of activity actually found for the nitrile group on ricinine from acetate-1-C^{14}. If glutamate-2-C^{14} was involved in a transamination reaction, the resulting α-keto-glutarate-2-C^{14} would give rise to carboxyl labelled succinate. Once again ricinine with a high proportion of activity in the nitrile

[3] No significance is attached to the greater incorporation of acetate-2-C^{14} than of acetate-1-C^{14}.

group would result. At present we are unable to explain the high proportion of activity found in the pyridone ring (Waller and Henderson, 1961) when succinate-1,4-C^{14} was fed to Ricinus. We hope to repeat this experiment in the near future.

Since both the 1 and 3 carbon atoms of propionate are able to supply the nitrile group of ricinine (Anwar et al., 1961), propionate is not a direct precursor of ricinine. The incorporation of propionate can possibly be explained by β-oxidation to malonate with subsequent decarboxylation to acetate.

Incorporation of succinate as suggested above leaves three other ring carbon atoms of ricinine to be accounted for. Until further degradations to isolate the ring carbons of ricinine are carried out, no firm conclusions can be reached as to whether or not glycerol is a direct precursor. Glycerol-1-C^{14} and glycerol-2-C^{14} were incorporated to the same extent as most of the other precursors listed in Table 1, but a large proportion of the activity was concentrated on the methyl groups which have already been shown (Dubeck and Kirkwood, 1952) to come from L-methionine-CH_3-C^{14}. The proportions of activity in the nitrile groups are as might be expected if some of the glycerol were phosphorylated and converted to acetate through glycolysis.

REFERENCES

Anwar, R.A., Griffith, T., and Byerrum, R.U., Federation Proceedings, 20, 374 (1961)
Beevers, H.A., Biochem. J., 66, 23P (1957)
Böttcher, B., Ber., 51, 673 (1918)
Dubeck, M. and Kirkwood, S., J. Biol. Chem., 199, 307 (1952)
Griffith, T., Hellman, K.P., and Byerrum, R.U., J. Biol. Chem. 235, 800 (1960)
Kornberg, H.L. and Beevers, H.A., Nature, 180, 35 (1957)
Ortega, M.J. and Brown, G.M., J. Biol. Chem., 235, 2939 (1960)
Pregl, F. 'Quantitative Organic Microanalysis', p. 156, 4th Ed., Blakiston Co., Philadelphia
Tamir, H. and Ginsberg, D., J. Chem. Soc., 2921 (1959)
Waller, G.R. and Henderson, L.M. Biochem. and Biophys. Research Comm., 5, 5 (1961)
Winterstein, E., Keller, J., and Weinhagen, A.B. Arch. Pharm., 255, 513 (1917)