SYNTHETIC PREFOLIC A

John C. Keresztesy and Kenneth O. Donaldson

National Institute of Arthritis and Metabolic Diseases
National Institutes of Health
Bethesda, Maryland

Received June 12, 1961

The existence of a new form of naturally-occurring folic acid, prefolic A, has been demonstrated (Donaldson and Keresztesy, 1959). It was isolated in essentially pure form as a barium salt from horse liver (Donaldson and Keresztesy, 1961). Enzymatic oxidation of prefolic A yielded two products, tetrahydrofolic acid (THFA) and formaldehyde (Donaldson and Keresztesy, 1961). We wish to report here the successful chemical synthesis of prefolic A.

Five hundred mg. of dl THFA (87%) prepared by the catalytic reduction of folic acid (O'Dell et al., 1947, and Kisliuk, 1957) were dissolved in 20 ml. M/3 phosphate buffer, pH 7.0, containing 0.3 ml. 37% formaldehyde solution. One gram of $NaBH_4$ was added and the reaction mixture was agitated at 37° by a stream of He. After one hour the excess $NaBH_4$ was destroyed by adding 0.4 ml. formaldehyde solution. Ascorbic acid 0.2 ml. of a 100 mg. per ml. solution (neutralized) was added. The reaction mixture was chromatographed on a DEAE cellulose column, 3.5 x 16 cm., prepared with 25 g. DEAE cellulose in 10% Na_2HPO_4 at room temperature (23°). The column was eluted with 10% Na_2HPO_4. Ten ml. fractions were collected. The u. v. absorption curves were made with a Cary Model 11M Recording Spectrophotometer.

Fractions #23 through #50 had the same absorption characteristics as prefolic A, max. at 290 mμ and min. at 245 mμ. After combining these fractions, 1.0 ml. 2-mercaptoethanol was added followed by 2.5 volumes of methanol. After chilling overnight at 0° the precipitated sodium phosphate was removed by filtration and washed with 200 ml. 80% methanol. The combined filtrate and wash was concentrated in vacuum, $T < 25^\circ$, to 80 ml. Twenty ml. of 20% $BaCl_2 \cdot 2H_2O$ was added and the precipitated barium phosphate removed by centrifugation followed by a water wash. To the combined solutions 2.5 volumes of absolute ethanol were added and after 3 hrs. at 0° the precipitated barium salt was recovered, washed and desiccated. It weighed 502 mg. This was extracted with three 2 ml. portions of 1% aqueous 2-mercaptoethanol at room temperature. The first purified barium salt was obtained by adding 0.1 volumes of methanol and chilling at 0° for 90 min. After recovery, washing and drying, this fraction (K61-61A) weighed 178 mg. On bringing the methanol concentration of the combined supernates and washes to approximately 50%, a second fraction (K61-61B) weighing 101 mg. was similarly recovered. An additional 27 mg. was obtained from the supernate. The total recovery was 306 mg. of barium salt.

The u. v. characteristics of K61-61A and -61B agreed with that of natural prefolic A. All showed a single maximum in pH 7.0 buffer at 290 mμ and a minimum at 245 mμ. In 0.1 N HCl all showed two maxima, 292 and 269 mμ. On standing overnight at 37° in 1% Na_2HPO_4, each developed an additional new maximum at 250 mμ, indicating conversion to prefolic AB (Donaldson and Keresztesy, 1961).

Table I gives a comparison of the microbiological properties which shows that the synthetic product is approximately 50% as active as the natural prefolic A within the limits of these assays. The synthetic prefolic A preparation contains the diastereoisomers derived from the starting dl-tetrahydrofolic acid.

Table I

Comparison of microbiological activity of natural and synthetic prefolic A per mg. of Ba salt

Source	Assay Organism	
	P. cerevisiae μg	L. casei μg
Natural	<2.5	675.0
Synthetic	<2.5	425.0
Natural + enzyme*	550.0	565.0
Synthetic + enzyme*	350.0	305.0

*The enzymatic conversion of prefolic A to tetrahydrofolic acid was carried out as described by Donaldson and Keresztesy (1961).

REFERENCES

1. Donaldson, K. O. and Keresztesy, J. C. J. Biol. Chem. 234, 3235 (1959).
2. Donaldson, K. O. and Keresztesy, J. C. Federation Proc. 20, 453 (1961).
3. Donaldson, K. O. and Keresztesy, J. C. Biochem. Biophys. Res. Comm. 5, 289 (1961).
4. Kisliuk, R. L. J. Biol. Chem. 227, 805 (1957).
5. O'Dell, B. L., Vandenbelt, J. M., Bloom, E. S., Pfeffner, J. J. J.A.C.S. 69, 250 (1947).