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# SHIKIMATE-DERIVED METABOLITES. 10.
# SYNTHETIC STUDIES ON THE BIOSYNTHESIS OF PARA-AMINOBENZOIC ACID.

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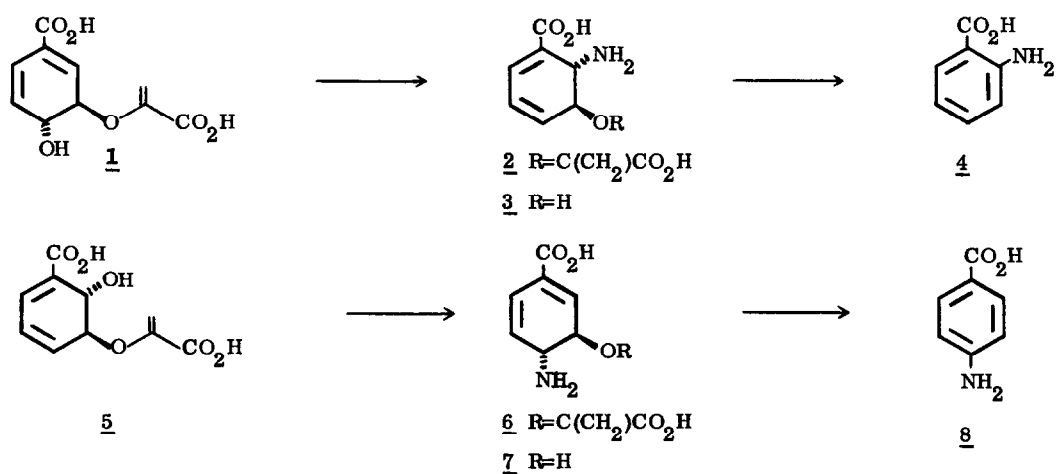
Summary: The total synthesis of trans-4-amino-3-hydroxycyclohexa-1,5-diene carboxylic acid 7, an hypothetical intermediate in the biosynthesis of p-aminobenzoic acid, has been achieved.

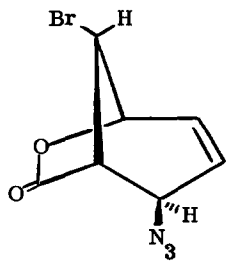
Chorismic acid 1 is the precursor of p-aminobenzoic acid 8 (PABA) and anthranilic acid 4, two substances which figure prominently in the biosynthesis of tryptophan, tetrahydrofolic acid and other metabolically important natural products.[2] Although labelling experiments have implicated glutamine as the principal nitrogen source for 4 and 8, the stepwise origin of these aminoacids from chorismate remains incompletely understood. Dardenne[3] and Haslam[4] have postulated a symmetric pattern of transformations linking chorismate and isochorismate 5 with 4 and 8, respectively, by parallel 1,6-addition-eliminations as pictured below. The characterization of 3[5] and its lactic acid ester oryzoxymycin[6] support the formation of anthranilate by this pathway, although the scheme remains controversial.[7] Structures 6 and 7, whose existence would support the complementary pathway to PABA, have not yet been detected as bona fide intermediates.



In this Letter we describe the first synthesis of trans-4-amino-3-hydroxycyclohexa-1,5-diene carboxylic acid 7, a conceivable in vivo progenitor of 8. Its chemical properties, especially its remarkable stability in acid and base, suggest that its isolation from the natural environment as a non-enzyme-bound intermediate may not be as formidable as once presumed.

Bicyclic dibromide 9[8] underwent clean $S_N2$ inversion upon exposure to NaBr in HMPA (rt, 18h) and afforded 10 in near quantitative yield [mp 151-152° (ether-$CH_2Cl_2$); NMR δ ($CDCl_3$) 6.19, 5.91 (2m, $H_D$, $H_E$, $J_{DE}$ = 10 Hz), 4.93 (broad s, $H_B$, $H_C$), 4.47 (s, $H_A$), 3.33 (d, $H_F$, $J_{EF}$ = 6 Hz)].[9] Substitution of the allylic bromide in 10 with $NaN_3$ (2 equiv., anhydrous DMSO, 80°, 2.5h) gave a 4.5 : 1 ratio of azides 11 and 12 which could be separated by silica gel chromatography (70% combined yield). Interestingly, 9 was transformed into 12 as the exclusive product by the same reaction conditions. Pure 11 [oil; NMR δ 6.25, 5.92 (2m, $H_D$, $H_E$, $J_{DE}$= 10 Hz, $J_{EF}$ = 7 Hz, $J_{CD}$ = 3 Hz), 4.74 (m, $H_B$, $J_{BC}$ = 3 Hz), 4.45 (s, $H_A$), 4.28 (t, $H_C$), 3.34 (d, $H_F$); IR $\lambda_{max}$ (film) 4.74, 5.60 μ ][9] was hydrogenated to 13 with Lindlar's catalyst[10] in 78% yield. This relatively unstable amine [hydrochloride salt: 204° (discoloration), 236-7° (d)] was immediately saponified using 3 equiv. NaOH in aqueous THF (rt, 4h). Concentration of the reaction mixture afforded crude 7, Na salt, in quite pure form, contaminated with ~5% PABA [NMR δ ($D_2O$) 6.43 (d, J=2 Hz, $H_A$), 6.30 (dd, J=3, 11 Hz, $H_E$), 5.84 (dd, J=1.5, 11 Hz, $H_D$), 4.31 (dd, J=2, 11 Hz, $H_B$), 3.53 (dd, J=3, 11 Hz, $H_C$)].

Aqueous solutions of this salt were found to be stable over a period of days at pH 10-11. Ion exchange chromatography (Amberlite IR 120H) furnished the zwitterionic aminoacid 7 as a pale yellow powder which could be further purified by reverse phase HPLC [55% yield; mp 180° (darken), 205° (d); NMR δ ($D_2O$) 6.50 (broad s, $H_A$), 6.47 (dd, $H_E$), 5.91 (dd, $H_D$), 4.57 (dd, $H_B$), 3.95 (broad dt, $H_C$); UV $\lambda_{max}$ 268 nm (ε 1730, $H_2O$); IR (KBr) $\lambda_{max}$ 2.92, 6.50, 7.20 μ; MS (CI) 156 (.3%, M+1)), 139 (base, $-NH_3$)]. The hydrochloride salt of 7 was also prepared [mp 168-172° (d); NMR δ ($D_2O$) 6.94 ($H_A$), 6.58 ($H_E$), 5.98 ($H_D$), 4.70 ($H_B$), 4.23 ($H_C$)]. NMR spectroscopy on this series of structures revealed, as expected, dramatic variations in the chemical shifts of ring protons next to the carboxyl and amino functions as these groups became electrostatically charged.



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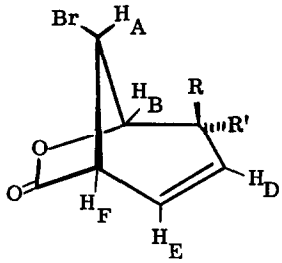
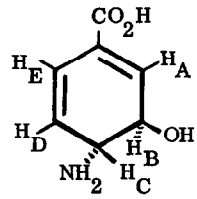


9 R = Br, R' = $H_C$

10 R = $H_C$, R' = Br

11 R = $N_3$, R' = $H_C$

13 R = $NH_2$, R' = $H_C$



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Aromatization of the hypothetical intermediate required forcing conditions and proved to be pH-dependent. When 7 was heated with excess NaOH (5 equiv., 65-70°, 5.5h), both m-hydroxy- and p-aminobenzoic acids were produced in a molar ratio of 1.8 : 1.[11] In contrast, only m-hydroxybenzoic acid arose from exposure to concentrated HCl (37% solution, 70-80°, 7.5h). While the remarkable chemical stability of this aminocyclohexadienol parallels that of its counterpart 3[5] in the proposed biosynthetic scheme, its behavior remains poorly understood at this time. From these observations it would appear that the *in vivo* transformation of 7 to PABA must involve precise enzymatic regulation. The ability of 7 to satisfy a requirement for PABA in *E. coli* auxotrophic mutants is presently being investigated.

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## REFERENCES AND NOTES


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