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GABACULINE: γ-AMINOBUTYRATE AMINOTRANSFERASE INHIBITOR OF MICROBIAL ORIGIN

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In the course of the studies on enzyme inhibitors produced by microorganisms, gabaculine was discovered in a culture filtrate of Streptomyces toyocaensis subsp. 1039 and it showed inhibition (10^{-8} - 10^{-9} mole/ml for I_{50}) of γ-amino-butyrate aminotransferase.

Herein we report the structural elucidation and synthesis of gabaculine.

Gabaculine, isolated as an amorphous powder, $[\alpha]_D = -454^{\circ}$(c=1, H_2O), has the formula $C_7H_9NO_2$ (M^+ 139.0642).

The ir spectrum of gabaculine shows an absorption of conjugated carbonyl group at 1650 cm^{-1} and the uv spectrum shows an absorption maximum at 275 nm (ε 8600) indicating the presence of a carbonyl chromophore conjugated with a dienyl group.

The nmr spectrum of gabaculine is shown in Fig. 1. The signals of an ABX system at δ 6.82 are assigned to the β-proton of an α,β-unsaturated carbonyl group. Coupled signals due to two protons at lower field (δ 6.06 and 6.47) are considered to be vicinal olefinic protons in a cis relationship (J=9.5 Hz) and a double doublet at higher field (δ 2.77) is attributed to the geminal methylene protons situated in an allylic position. A multiplet at δ 4.11 is assigned to a hydrogen on the carbon attached to an amino or hydroxyl group. The coupling features in the nmr spectrum has been confirmed by the application of double resonance techniques.

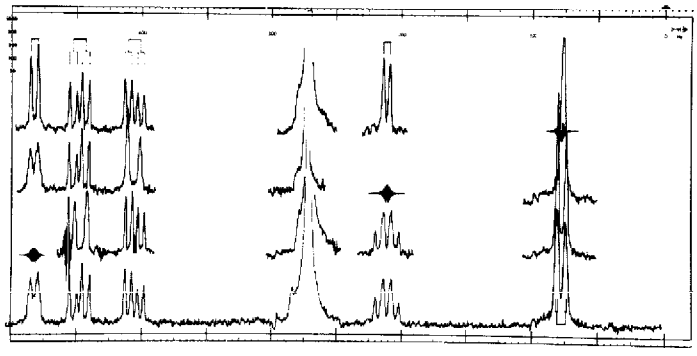
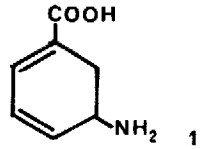
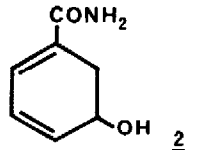


Fig. 1

On the basis of spectroscopic data, two possible structures (1 and 2) are proposed.



1

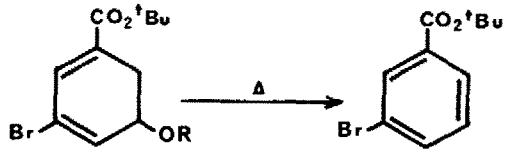


2

The mass spectrum of gabaculine shows the same abundant peaks as those of benzoic acid except for the molecular ion and base peak (m/e 94, M^+-COOH).

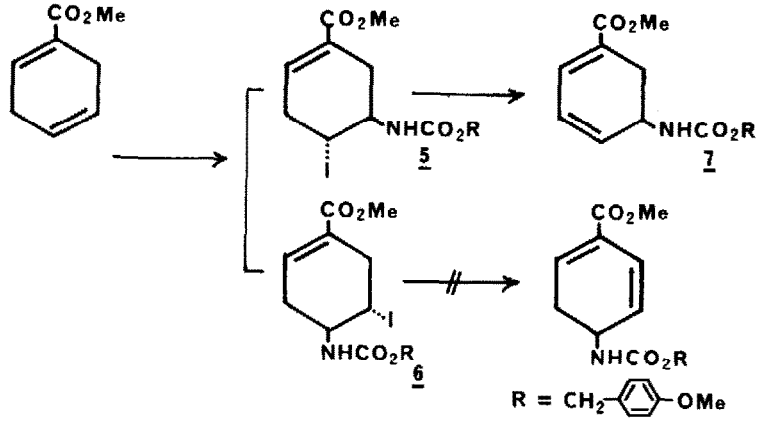
The functional groups of gabaculine are a carboxyl group, which was esterified with diazomethane, and an amino group, which gave a positive ninhydrin color test. Consequently the structure of gabaculine was determined as 1.

It seems surprising that gabaculine could be isolated as a stable compound, because it has been reported that dihydrophenol (3) and its acetate (4) were relatively unstable and these compounds could not be purified without decomposition.[1] Thermal decomposition of 3 or 4 yields tert-butyl m-bromobenzoate.



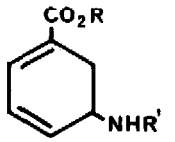
3 R = H
4 R = Ac

The synthetic approach for gabaculine was designed to avoid thermal reaction conditions and an extensive work-up.



Electrophilic addition of iodo-isocyanate in CH_2Cl_2[2] to methyl 2,5-dihydrobenzoate, followed by treatment with p-methoxybenzylalcohol in the presence of dibutyltindilaurate,[3] afforded the carbamate consisting of approximately equal amounts of the pair of isomers 5 and 6 (estimated from the nmr spectrum).

Dehydroiodonation of 5 with DABCO in acetone at room temperature gave a mixture of dienyl carboxylate (7) and unreacted carbamate (6). The Rf values of 5, 6, and 7 on TLC were exactly identical. Without purification of 7, treatment of the mixture (6 and 7) with CF_3CO_2H in anisole at room temperature resulted a crystalline precipitate of 8 as the trifluoroacetic acid salt, mp. 142-3°.



8 R = Me R' = H

9 R = H R' = $CO_2{}^tBu$

Reaction of 8 with a base such as $Ba(OH)_2$ formed benzoic acid and a small amount of dl-gabaculine.

The carboxylic acid (9), mp. 148-50°, could be obtained from 8 by protecting the amino group as the tert-butyloxycarbonyl derivative and subsequent hydrolysis with NaOH at room temperature. Removal of the protective group of 9 was accomplished by treatment with dil. HCl at room temperature and dl-gabaculine hydrochloride was obtained as needles of mp. 198-200°.

dl-Gabaculine, mp. 196-7°, was eluted with NH_4OH from the ion-exchange column (SP Sephadex C-25).

The identity of the racemic form with the natural one was confirmed by spectroscopic data (ir, uv, nmr, mass), TLC, and aminoacid analysis.

It should be mentioned that dl-gabaculine has half the activity of inhibiting γ-aminobutyrate aminotransferase in comparison with that of the natural form.

References

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