Racemic Guaifenesin Preparation by *in vivo* Williamson ether synthesis and FTIR, NMR, HPLC and GCMS spectral analysis

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Abstract
The preparation of racemic guaifenesin (3-(2-methoxyphenoxy)-1,2-propanediol by a Williamson ether synthesis reaction was well-suited towards the reaction mechanisms, spectroscopic analysis and pharmaceutical synthesis of enantioselective compound. Analysis of the reaction products includes NMR, HPLC, GC techniques that involve comparison of the reaction products to known standards.

The most characteristic peaks in an IR spectrum are those associated with O-H bonds (strong, broad peaks, 2968.80 cm-1) and the carbonyl group (C=O, strong peaks 1379.10 cm-1). Identification of synthesized product structure chemical shift occurred at 3.2 ppm indicating the presence of methyl compound in structure of guaifenesin and the molecular mass synthesized product was found to be 198g/mol for guaifenesin compound with the CAS No.of 93-14-1.

Keywords: Scanning Electron imaging, Quantitative Spectral analysis, YEPD, CMA, Malt extract medium, Yeast isolates.

Introduction

One of the main misconceptions nowadays in modern life is the difference between "natural" products and a "synthetic" one. In an attempt to return to more environmental and health-conscious lifestyle, there is a distinct chemistry in the natural world than that used in modern pharmaceutical industry. Commercial products deemed natural are labeled safe, healthy and green, while those from pharmaceutical industry are labeled in less complimentary ways. Nature through billions of years of chemical experimentation (mostly through living things) has economized the production of thousands of compounds from simple compounds in the environment for use by life.

Furthermore, once these compounds are used, they are degraded into molecules that are readily used by other living things, often forming closed cycles. With a heritage of only mere 150 years, it is difficult to expect modern synthetic chemistry to be as efficient or viable economical. In many cases the urgent need for a specific chemical compound may preclude any thought given to by-products. The final step of any human made chemical process is to close the loop and optimize the process by Williamson ether synthesis.[6,16]

Alexander Williamson developed this reaction in the year 1850. This is a reaction which uses the deprotonated alcohol and an organohalide to form an ether. Williamson ether synthesis usually occurs as an S_N2 reaction of a primary alkyl halide with an alkoxide ion. The ether produced by this reaction has more number of carbon atoms than that of starting material and thus it possesses a complex structure as in fig. 1. Thus Williamson ether synthesis takes a special place in organic chemistry.[2,12]

Generally, the Williamson synthesis utilizes a alkoxide nucleophile or phenoxide (oxygen is small and has a full negative charge) working on a (usually) primary alkyl halide (Cl, I or Br leaving groups) substrate. Although SN^2 reactions are promoted by polar aprotic solvents, the high nucleophilicity of the oxygen nucleophile and primary substrate allow the reaction to occur in protic solvents at higher temperatures (i.e. greater number of collisions per unit time). Commercial synthesis of guaifenesin is the foremost convenient route to use the phenoxide (from 2-methoxyphenol) nucleophile as it is easily generated by deprotonation.

Additionally, this end of the ultimate ether could not be used as a substrate for SN^2 as a SP^3 carbon would bear the leaving group. The substrate can be the primary alkyl halide R-3-Chloro-1,2- propanediol. SN^2 reaction means substitution, nucleophilic, bimolecular. In these reactions, an appropriate suitable nucleophile collides with an unhindered SP^3 carbon center bearing an honest leaving group.

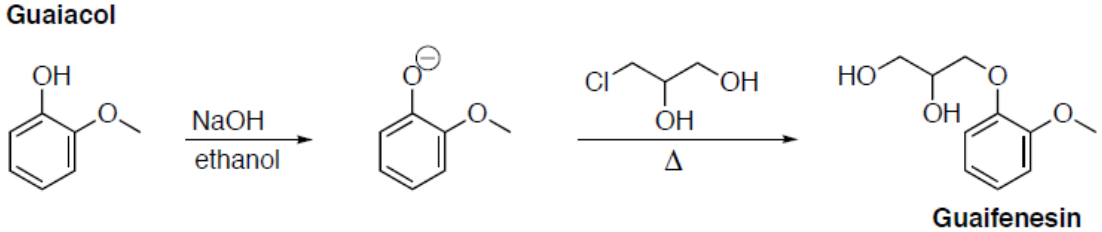

Fig. 1: Williamson ether synthesis mechanism

The two alcohols get reacted together after which one of them is interchanged as a leaving group (tosylate). The alkylating agent is preferred as a primary product and alkoxide obtained could be considered as secondary product. Sulfonate ester produced during this reaction is the leaving group.[9] Guaifenesin is a white to slightly gray, crystalline powder, derived from the resin of guaiacum trees with IUPAC name (RS)-3-(2-methoxyphenoxy) propane-1,2-diol. It is soluble in water, freely soluble in alcohol, chloroform and propylene glycol and sparingly soluble in glycerin.

Guaifenesin, the glyceryl ether of guaiacol, is a component of numerous cough and cold preparations available worldwide termed as an expectorant. This medication is most commonly used to loosen mucus and phlegm and eventually clears the symptoms of congestion resulting from a cold or allergy.[4] It works by thinning mucus and phlegm in the body and the thinning action makes it easier for the body to expel excess mucus and phlegm, generally through coughing or the blowing of the nose.[5]

Material and Methods

Preparation of Guaifenesin *in vivo* (Williamson ether synthesis)

Materials: The glasswares used are: round bottomed flask, Pasteur pipette, graduated cylinder, glass funnel, beakers, separation funnel and for instrumental setups such as ring stand , stirrer/ hot plate, heating mantle, reflux condenser, rotary evaporator, vacuum filter, ice bath, Buckner funnel. Reagents and solvents required are: 95% ethanol,25% of NaOH solution, 5.98% of 95% ethanol, drying agent (magnesium sulphate), ethanol, methanol, ice cold hexane, ethyl acetate, all purchased from Himedia.

Williamson ether synthesis: Guaifenesin was synthesized using Williamson ether synthesis procedure in a grease necked 100ml round bottomed flask, attached to ring stand, set over stirrer. 15ml of 2-methoxy phenol solution (1.33M in 95% ethanol) was added; 4ml of 25% NaOH solution was added to the same round bottomed flask with a separate graduated cylinder with the aid of glass funnel. The heating mantle was fitted beneath the flask. A reflux condenser on the flask was attached to the hose tubes to ensure inlet and outlet of water supply. Heating mantle that was connected to variac was switched on now and the reaction mixture was allowed to stirr continuously for 15 minutes.

Results and Discussion

Preparation of Racemic Guifenesin *in vivo* (Williamson ether synthesis): Williamson ether synthesis was performed to synthesise guaifenesin. The solid resultant product retained in the Buckner funnel after vacuum filtration was transferred to a watch glass and allowed to dry on watch glass in the hood. After product was dried completely, it was weighed and the mass was recorded. The % yield was calculated and found as 89.7%. Melting point of the solid product was determined. The melting point of standard guaifenesin and synthesized guaifenesin was determined 78.5-79°C and the resulting data were compared to that of the melting point range as reported in Pharmacopeia.

Thin Layer Chromatography: The product (guaifenesin) obtained from Williamson ether synthesis analyzed by TLC against authentic (±) Guaifenesin.50:50 ration of hexane: ethyl acetate was used as a developing solvent for TLC and distance travelled by the analyte was detected using UV light. The R_f value was calculated and recorded The synthesized product showed Rf values as synthesized guaifenesin 0.86, standard guaifenesin as 0.86 (fig.2). In visible light the spots show light pale yellow color while under UV light the spots showed fluorescent greenish yellow color.

FTIR Spectrum: FTIR was an indispensible analytical technique for identification of active pharmaceutical ingredients (API). FTIR exhibited data related to characteristic peaks that indicate presence of functional group in the product. The peaks observed for the product guaifenesin (fig. 3**)** at 2968.80 cm^{-1} 2924.09 cm^{-1} are characteristic feature of C-H as seen in alkenes, 2868.51 cm^{-1} is the characteristic feature of O-H stretch as seen in alcohols and phenols. The peaks at 1463.97 cm^{-1} may the characteristic feature of C-C stretch as in aromatics, 1379.10 cm^{-1} as seen in C-C stretch as in aromatics, 885.3 cm^{-1} is the characteristics of alkenes, 726.3 cm^{-1} is the characteristics of aromatics and 428.20 cm^{-1} is the characteristic feature of C-Cl as seen in alkyl halides.

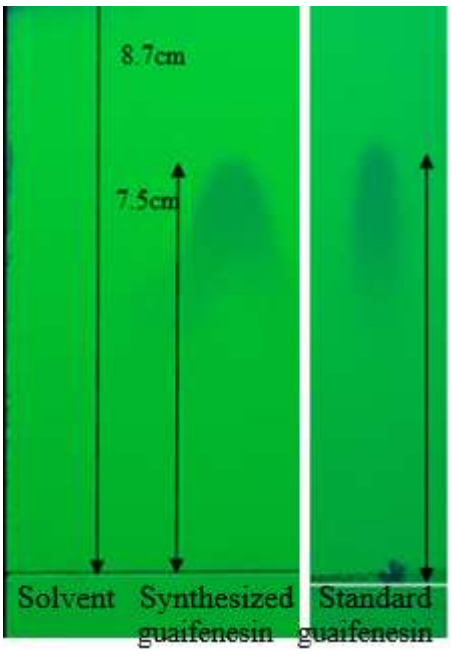

Fig. 2: Seperation of analyte by TLC Lane 1 Product Lane 2-guaifenesin

Nuclear Magnetic Resonance Spectrum: The NMR spectrum of standard guaifenesin and the product synthesized from MGR5 and MGR6 was given in fig. 4. Various peaks were assigned to the spectrum. In ^1H spectrum

the chemical shifts (ppm) at 7.1, 7.4, 7.9 and peaks at 3.5, 4.7 and 2.1 indicate aromatic compounds present in the given compound. In the synthesized product chemical shift at 3.2ppm indicates the presence of methyl compound in structure of guaifenesin.

High performance Liquid Chromatography: Guaifenesin was determined and quantified with the help of high performance liquid chromotography in isocratic condition. The fig.5 expresses the separation of guaifenesin product. The appearance of peak areas in the HPLC chromatogram was based on retention time of the analytes injected. The areas of the peak and related quantifications are given in the table 1. The sharp peaks in HPLC indicate the presence of guaifenesin. The retention time peak was 2.47min, concentrations were 98 and 100%. Previous report states that stability of the guaifenesin drugs. Appearance of the parent drug (guaifenesin) peak (after 5.944 min) and disappearance of the prodrug peak (10.961, 14.999, 14.142 min) was monitored to determine the rate of conversion for each of the 3 prodrugs.[15]

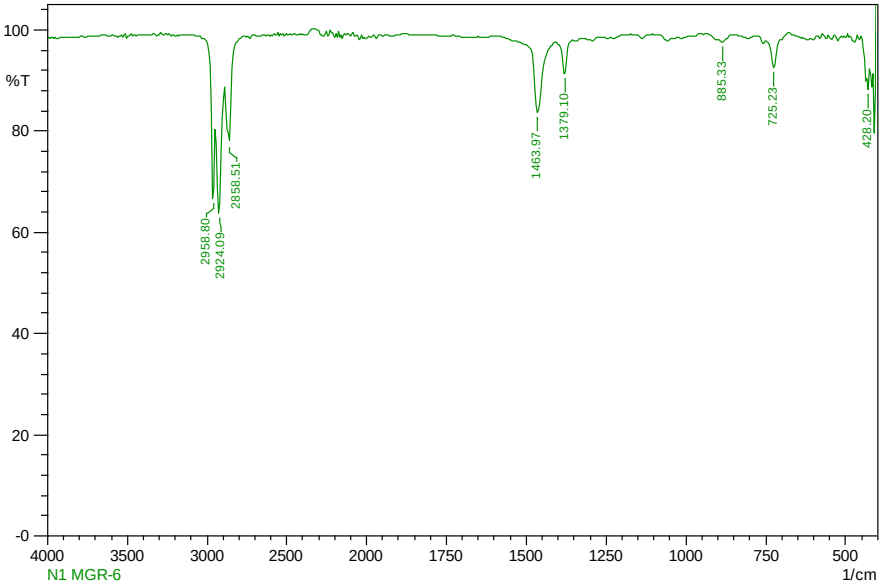

Fig. 3: IR Spectra of synthesized guaifenesin

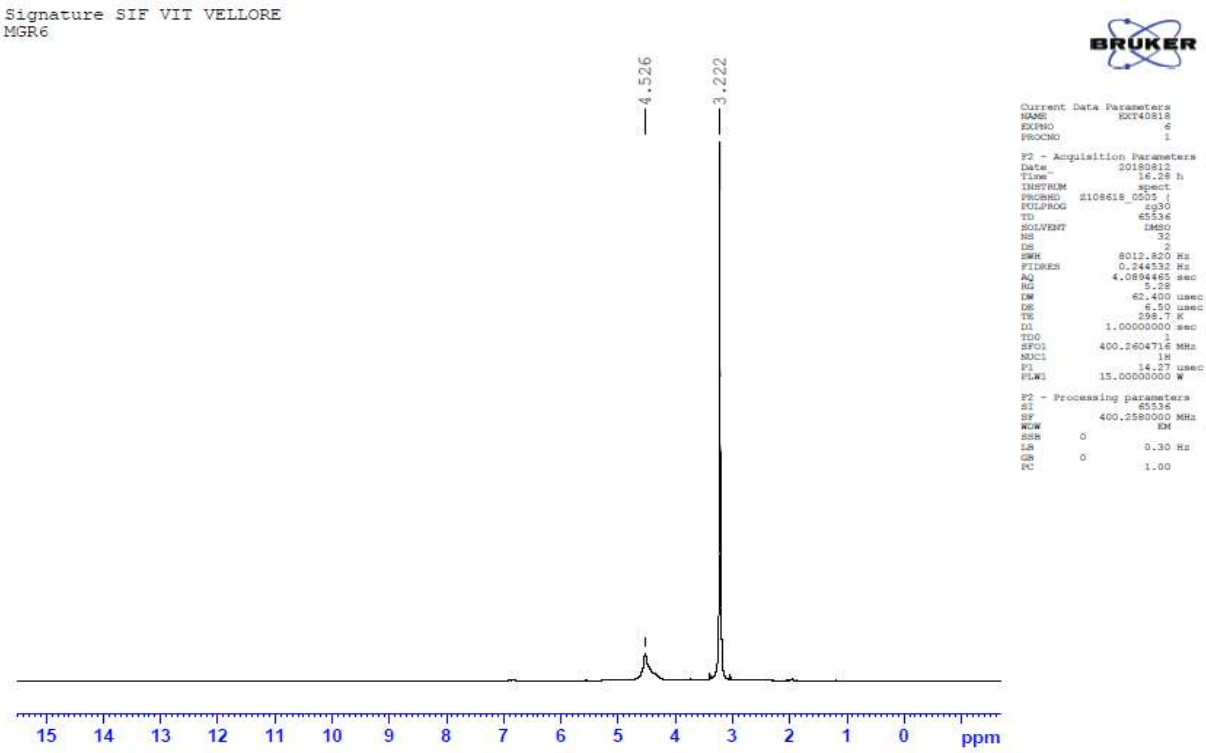

Fig. 4: NMR Spectra of synthesized guaifenesin

Table 1
Characteristic feature of HPLC chromatogram of standard guaifenesin and synthesized guaifenesin

Index No.	Compound	Retention Time(min)	Peak area (%)	Concentration	Theo. plate	Tail factor
1.	Standard guaifenesin	8.167	89.60%	34.57	89.6653	111200.27
2.	MGR 5 guaifenesin	9.569	87.36%	4.05	87.3650	111496.99

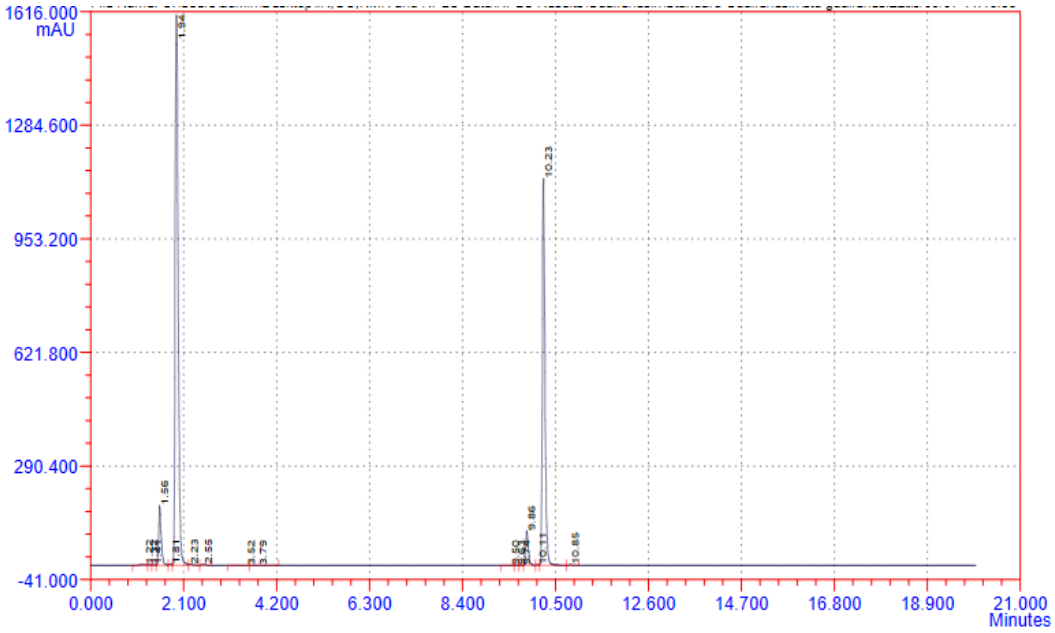

Fig. 5: HPLC peaks of synthesized guaifenesin

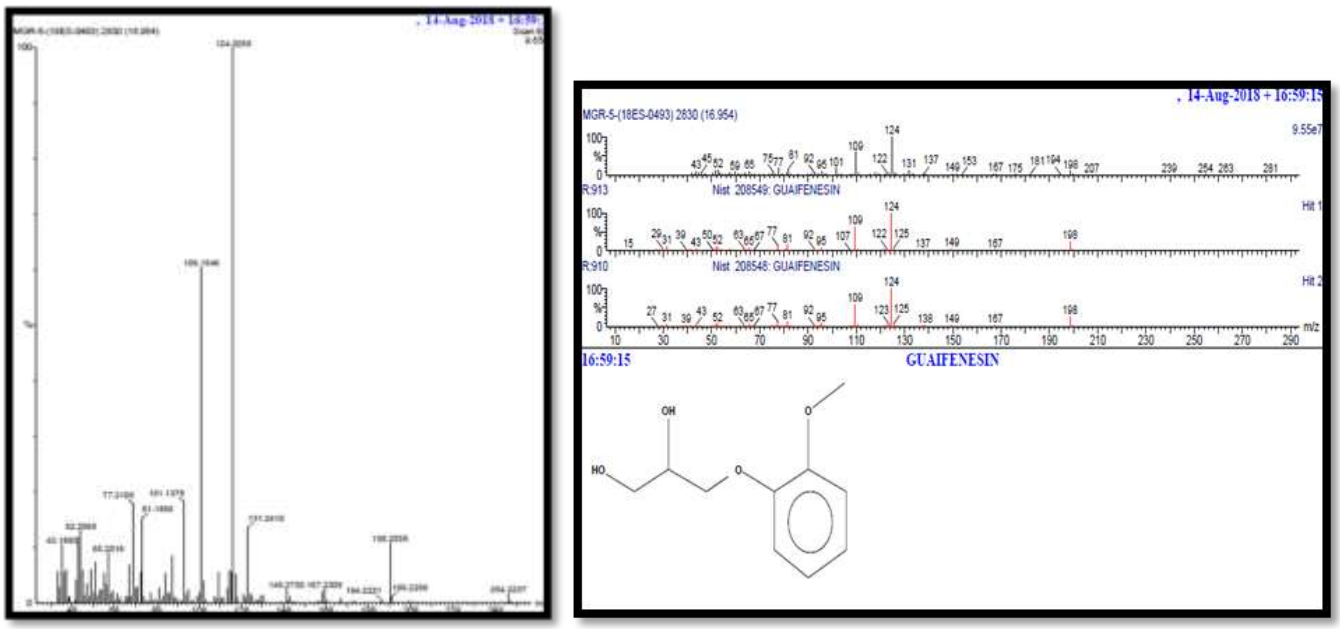

Fig. 6: GC-MS Spectra of a) synthesized guaifenesin b) Comparison with guaifenesin standard

Conclusion

It has been now found that by employing (R)-3-Chloro 1,2-propanidol to react with the conjugate base of 2 methoxyphenol via SN^2 reaction through Williamson ether synthesis, yield was calculated and found to be 89.7%.It can be concluded that nucleophilic anion reacts with the primary alkyl chloride carbon of 3-chloro-1,2-propanediol to give the highest target product through Williamson ether synthesis. The active guaifenesin so obtained may readily be further enriched in the predominate enantiomer by recrystallization.

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