International Journal of Chemistry and Applications.
ISSN 0974-3111 Volume 3, Number 3 (2011), pp. 229-235

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Synthesis and Antibacterial Activities of Citral Derivatives

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Abstract

Citral, a bioactive compound in Palmrose oil, although known to have antibacterial effect, has not been used in current drug research. Optimization of the lead structure could enhance the utility of this compound. Hence citral was modified using natural amino acids to produce the Schiff's base, a potential pharmacophore and the corresponding amines. In this study, we report the synthesis of 9 Schiff's bases, and the corresponding amines (Reduced product) and their antibacterial activities.

Introduction

Natural Products were the basis of the first pharmaceutical Practice and they continue to play an important role in modern chemotherapy. They are the most successful source of drug leads. The global scenario is now changing towards the use of these non-toxic plant products having traditional medicinal use emphasizing the development of modern drugs from natural sources for the control of various deceases. Although herbal medicines in form of crude extracts have been used from time immemorial, modern drugs can be developed after extensive investigation of its bioactivity, mode of action, pharmacotherapecutics, and toxicity and after proper standardization and clinical trials. Citral, or 3,7-dimethyl-2,6-octadienal or lemonal, is either of, or a mixture of, a pair of terpenoids with the molecular $C_{10}H_{16}O$. The two compounds are double bond isomers. It has strong anti-microbial qualities, and pheromonal effects in insects. Citral seem to be a very good synthon for the development of active molecules. In the present study, an attempt has been made for the synthesis of novel compounds from citral. Citral is the primary component of the essential oil of lemongrass, constituting well over half the total oil content. Citral is one of the many chemicals giving lemongrass its strong lemony aroma, and it is also found in lemons and a number of other lemon-scented herbs. Citral has been studied for its potential to treat cancer, due to its ability to induce cell death (apoptosis) in

cancer cells. Cancer cells are able proliferate in part by deactivating the cell death program, which normally causes damaged or mutated cells to self-destruct. The work on citral inducing cell death in cancer cells comes from in vitro experiments (controlled laboratory experiments, as in test tubes or petri dishes), and these results have not been verified in human or animal studies. It is not known the degree to which these properties would actually carry through into humans drinking lemongrass tea or taking extracts of lemongrass as herbal supplements. In this study, we report the synthesis of 9 Schiff's bases, and the corresponding amines (Reduced product) and their antibacterial activities.

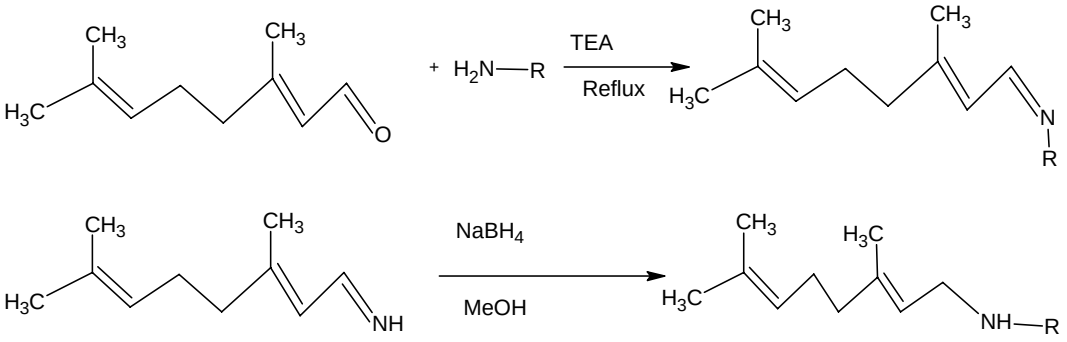
Materials and Methods

Fresh Citral was purchased, and purified by distillation. The purity was confirmed by GC, and NMR. Amino acid esters, solvents were purchased from Sigma-Aldrich and were used as supplied. Thin-layer chromatography (TLC) was performed on 0.25 mm pre coated silica gel 60 F254 aluminum sheets and column chromatography on silica gel 60 (0.063-0.2 mm) as well as silica gel 60 (<0.063 mm), products of Merck & Co. (Darmstadt, Germany). The C^{13} & H^{1}NMR spectra were recorded, with a Bruker (500 MHz) spectrometer, with TMS as internal standard.$CDCl_3$ was used as the solvent.

Experimental

The Azomethine was obtained by refluxing citral with corresponding amino acid methyl ester in methanol medium, using triethyl amine as the catalyst. The amine-derivative azomethine was also made in the same fashion. Azomethine obtained was further reduced to corresponding amines by treating the with sodium borohydride in methanol medium. The scheme is given below

Scheme:



R = CH_3, $C_6H_5CH_2$, $CH_2CH(CH_3)_2$, $CH(CH_3)$ CH_2CH_3, CH_2SH, $CH_2CH_2SCH_3$, $CH(CH_3)_2$

Synthesis of azomethine from citral

In a typical procedure, weighed quantities of citral and amino acid methyl ester

hydrochloride (*Sigma Aldrich standard, Buchs, Germany)* in the ratio 1:1 was refluxed in methanol medium with stoichiometric amount of triethyl amine (TEA) . The reaction was monitored by TLC ((60/120 mesh, Sigma Aldrich, Germany,. After 6-8 h methanol was distilled off in a rotary evaporator and the residue was subjected to column chromatography. Pure Schiff's base compound was eluted in 8:2 methanol:chloroform mobile phase. The purity and yield of the synthesized schiff's bases are given in table 1 and Fig 1.

Azomethine was also synthesized using different hydroxyl amines, following the above procedure.(Table2)

Synthesis of amines from azomethine

In a typical procedure, weighed quantities of synthesized Azomethine and sodium borohydride (Sigma Aldrich standard, Buchs, Germany*)* in the ratio of 1:1 was allowed to react under stirring in methanol medium, at room temperature. The reaction was monitored by TLC (60/120 mesh, (Sigma Aldrich, Germany). After 2-3 h, the reaction was quenched with dil.HCl. Methanol was distilled off in a rotary evaporator and the residue was subjected to column chromatography. Pure compound was eluted in 6:4 methanol:chloroform mobile phase. The purity and yield of the synthesized amines are given in table 3 and Fig 2.

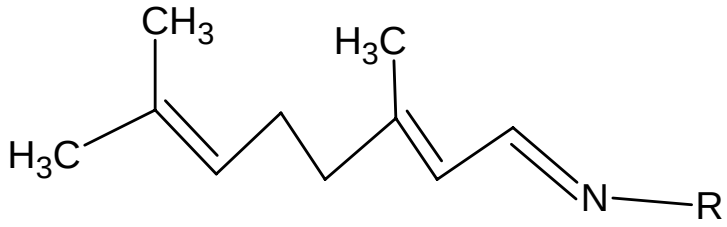


Figure 1: Structure of azomethine.

Table 1: Azomethine yield obtained from reaction of citral with methyl esters of different amino acids.

Compound no	Corresponding amino Acid used	Azomethine Yield (%)
1a	Glycine	86
2a	Alanine	79
3a	Phenyl alanine	89
4a	Leucine	79
5a	Isoleucine	85
6a	Cysteine	89
7a	Methionine	90
8a	Valine	75
9a	Threonin	76

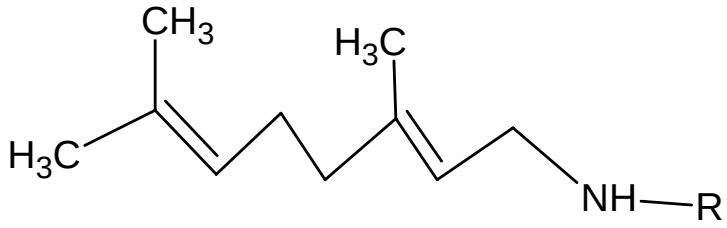


Figure 2: Structure of a typical amines derived from azomethine.

Table 3: Yield of amines obtained from reduction of azomethine obtained from different amino acids.

No	Corresponding aminoAcid/amine used	Yield (%)
1b	Glycine	86
2b	Alanine	89
3b	Phenyl alanine	89
4b	Leucine	74
5b	Isoleucine	75
6b	Cysteine	89
7b	Methionine	90
8b	Valine	75
9b	Threonin	76

In-vitro antibacterial activity

The antimicrobial activity (bacteria) of the compounds was evaluated by agar well diffusion method (Ahmad and Beg, 2001). All the microbial cultures were adjusted to 0.5 McFarland standards, which is visually comparable to a microbial suspension of approximately 1.5 $\times 10^8$ cfu/ml (Andrews, 2001). 20ml of Muller Hinton agar media was poured into each petriplate and plates were swabbed with 100 µl inocula of the test microorganisms and kept for 15 min for adsorption. Using sterile cork borer of 8mm diameter, wells were bored into the seeded agar plates and these were loaded with a 100 µl volume with concentration of 10 mg / ml Aloin based compounds reconstituted in the dimethylsulphoxide (DMSO). All the plates were incubated at 37°C for 24 hrs for Prediffusion. Antimicrobial activities of samples were evaluated by measuring the zone of growth inhibition against the test organisms with zone reader (Hi Antibiotic zone scale). The medium with DMSO as solvent was used as a negative control whereas media with ampicillin (10mg/ml) was used as positive control. The experiments were performed in duplicates.

Results and Discussion

The schiffs base obtained by refluxing citral and amino acid methyl ester hydrochloride in the ratio 1:1 was refluxed in methanol medium with stoichiometric

amount of triethyl amine (TEA) . Various Schiffs base derivatives has been made using different amino acid methyl ester. The imine esters further reduced to corresponding amine ester and evaluated the bacterial activity. The *in vitro* antibacterial activity of the synthesized compounds in DMSO against medically important Gram positive and Gram negative bacteria is shown in Table

Table: The *in vitro* antibacterial activity of the synthesized Citral modified compounds (10 mg/ml).

Sample Name	Inhibition zone (mm/100µl)		
	E.coli	*P.aureus*	*K.pneumonia*
Control (ampicillin)	22	20	23
Citral	20	18	19
1a	23	14	19
2a	15	18	15
3a	20	---	22
4a	28	19	25
5a	30	23	30
6a	31	19	29
7a	31	12	27
8a	19	17	11
9a	20	19	22
1b	----	14	23
2b	19	28	19
3b	24	---	27
4b	31	27	25
5b	32	23	35
6b	34	29	29
7b	32	22	27
8b	25	20	18
9b	20	23	25

The striking feature observed from the table is that the Schiff bases possess slightly increased activity than the parent citral molecule. The enhanced activity shows promise for application in various antimicrobial treatment.

Supporting data

Compound 1a: H^1NMR (500MHz,$CDCl_3$) δ (ppm): 4.37,3.59, 2.44, 2.05-2.04, 1.29-1.65, 0.88-0.91 . C^{13}NMR (500MHz,$CDCl_3$) δ (ppm):165.7, 151.3,132.6, 129.9, 60.8, 44.3, 32.4, 32.0, 30.1, 29.8, 29.6, 27.6, 27.3, 23.1, 23.0, 22.7, 17.5. MS (EI) M+ = 223.3

Compound 2a: H^1NMR (500MHz,$CDCl_3$) δ (ppm): 4.67,3.19, 2.44, 2.05-2.04, 1.29-1.65, 0.88-0.91 . C^{13}NMR (500MHz,$CDCl_3$) δ (ppm):159.7, 151.2,132.6, 126.9, 59.8, 44.3, 32.4, 32.0, 30.1, 29.8, 29.6, 27.6, 27.3, 23.1, 23.0, 22.7, 17.5. MS (EI) M+ = 237.3

Compound 3a: H^1NMR (500MHz,$CDCl_3$) δ (ppm): 5.17,3.32, 2.54, 2.15-2.04, 1.29-1.65, 0.88-0.91 . C^{13}NMR (500MHz,$CDCl_3$) δ (ppm):162.7, 152.3,132.6, 126.9, 60.2, 44.3, 32.4, 32.0, 30.1, 29.8, 29.6, 27.6, 27.3, 23.1, 23.0, 22.7, 17.4. MS (EI) M+ = 313.1

Compound 4a: H^1NMR (500MHz,$CDCl_3$) δ (ppm): 4.23,3.71, 2.34, 2.15-2.04, 1.29-1.65, 0.88-0.91 . C^{13}NMR (500MHz,$CDCl_3$) δ (ppm):165.7, 150.3,131.6, 129.9, 59.8, 44.3, 32.4, 32.0, 30.1, 29.8, 29.6, 27.6, 27.3, 23.1, 23.0, 22.7, 17.5. MS (EI) M+ = 279.6

Compound 5a: H^1NMR (500MHz,$CDCl_3$) δ (ppm): 4.21,3.18, 2.74, 2.05-2.04, 1.29-1.65, 0.88-0.91 . C^{13}NMR (500MHz,$CDCl_3$) δ (ppm):162.7, 151.5,134.6, 129.9, 60.8, 44.3, 32.4, 32.0, 30.1, 29.8, 29.6, 27.6, 27.3, 23.1, 23.0, 22.7, 17.5. MS (EI) M+ = 281.3

Compound 6a: H^1NMR (500MHz,$CDCl_3$) δ (ppm): 4.36,3.53, 2.34, 2.05-2.04, 1.29-1.65, 0.88-0.91 . C^{13}NMR (500MHz,$CDCl_3$) δ (ppm):165.6, 151.3,132.6, 129.9, 60.8, 44.3, 32.4, 32.0, 30.1, 29.8, 29.6, 27.6, 27.3, 23.1, 23.0, 22.7, 17.5. MS (EI) M+ = 267.5

Compound 7a: H^1NMR (500MHz,$CDCl_3$) δ (ppm): 4.17,3.63, 2.34, 2.05-2.04, 1.29-1.65, 0.88-0.91 . C^{13}NMR (500MHz,$CDCl_3$) δ (ppm):165.7, 151.3,132.6, 129.9, 60.8, 44.3, 32.4, 32.0, 30.1, 29.8, 29.6, 27.6, 27.3, 23.1, 23.0, 22.7, 17.5. MS (EI) M+ = 303.1

Compound 8a: H^1NMR (500MHz,$CDCl_3$) δ (ppm): 4.39,3.19, 2.32, 2.05-2.04, 1.29-1.65, 0.88-0.91 . C^{13}NMR (500MHz,$CDCl_3$) δ (ppm):165.7, 151.3,132.6, 129.9, 60.8, 44.3, 32.4, 32.0, 30.1, 29.8, 29.6, 27.6, 27.3, 23.1, 23.0, 22.7, 17.5. MS (EI) M+ = 268.9

Compound 9a: H^1NMR (500MHz,$CDCl_3$) δ (ppm): 4.37,3.59, 2.44, 2.05-2.04, 1.29-1.65, 0.88-0.91 . C^{13}NMR (500MHz,$CDCl_3$) δ (ppm):165.7, 151.3,132.6, 129.9, 60.8, 44.3, 32.4, 32.0, 30.1, 29.8, 29.6, 27.6, 27.3, 23.1, 23.0, 22.7, 17.5. MS (EI) M+ = 265.4

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