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Synthesis, Characterization and Antibacterial Activity of mono-, di- and tri-tosylate of Glycerol

(Sintesis, Pencirian dan Aktiviti Antibakteria Gliserol mono-, di- dan tri-tosilat)

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ABSTRACT

Glycerol is a valuable co-product from oleochemical industry such as from fatty acid and biodiesel production. By having three hydroxyl groups in its molecule, glycerol can undergo chemical modifications that lead to many possible applications. This paper reports the tosylation process of glycerol with para-*toluenesulfonyl chloride (*p-*TsCl). Reaction of glycerol with* p-*TsCl in the presence of a base produced mono-, di- and tri-tosylate of glycerol even though the reaction was carried out at the mole ratio of 1.2:1.0 of glycerol to* p-*TsCl. The compounds were successfully isolated and characterized. Mono-, di- and tri-tosylate of glycerol exhibited inhibitory activity against* Staphylococcus aureus *(gram positive bacteria) and* Pseudomonas aeruginosa *(gram negative bacteria).*

Keywords: Antibacterial activity; glycerol; tosylate

ABSTRAK

Gliserol merupakan produk sampingan berharga yang diperoleh daripada industri oleokimia seperti daripada pengeluaran asid lemak dan biodiesel. Dengan mempunyai tiga kumpulan hidroksil dalam molekulnya, gliserol boleh menjalani pengubahsuaian kimia yang membawa kepada banyak aplikasi yang mungkin. Kertas ini melaporkan proses pentosilan gliserol dengan para-*toluenesulfonil klorida (*p-*TsCl). Tindak balas gliserol dengan* p-*TsCl menghasilkan gliserol mono, di- dan tri-tosilat walaupun ia telah dijalankan pada nisbah mol 1.2: 1.0 gliserol kepada* p-*TsCl. Sebatian tersebut berjaya dipisah dan dicirikan. Gliserol mono-, di-dan tri-tosilat mempamerkan aktiviti antibakteria terhadap* Staphylococcus aureus *(bakteria gram positif) dan* Pseudomonas aeruginosa *(bakteria gram negatif).*

Kata kunci: Aktiviti antibakteria; gliserol; tosilat

INTRODUCTION

Glycerol is non-toxic, stable under most conditions, easily digested and environmentally safe (Jungermann & Sonntag 1991). Although it can be produced by chemical synthesis, natural glycerol derived from oils or fats is preferred by today's customers and consumers. Naturally occurring glycerol is in the form of glyceride (Jungermann & Sonntag 1991). It occurs in combined form in all animal and vegetable fats and oils. It is usually present as triglycerides (also called triacylglycerols). Triglycerides are esters of fatty acids with glycerol where typically the fatty acids are different. That means different fatty acids can be attached to one glycerol backbone. Glycerol also occurs naturally in all animal and vegetable cells in the form of lipids such as lecithin and cephalins (Knothe et al. 2005).

The majority of commercially available glycerol results from the purification of the co-product obtained from oleochemical industry. Therefore, glycerol is a valuable co-product from oleochemical industry such as production of fatty acids, biodiesel and soap manufacturing. This is a good sign for oleochemical industry since the oleochemical-based product is claimed to be more environmentally friendly and it comes from renewable resources compared to petroleum-based product.

Glycerol is a polyhydric alcohol and by having three free hydroxyl groups, it can undergo many chemical reactions to form important chemical compounds and some of the compounds could be used to produce other derivatives. It has been discovered that the hydroxyl group of alcohols can be transformed into a good leaving group by replacing the hydroxyl group with tosyl or mesyl group. Treatments of alcohols with *para*-toluenesulfonyl chloride (*p*-TsCl) have been widely investigated (Brown et al. 1967; Lee et al. 2000; Tipson 1944; Wang et al. 2003). The tosylation of alcohols with *p*-TsCl in the presence of a catalyst under solvent-free conditions was also reported (Kazemi et al. 2007; Razieh et al. 2006). Besides tosyl group, mesyl group can also be introduced to the alcohols by allowing the alcohols to react with methanesulfonyl chloride (Jung & Shaw 1980).

Tosylates contain an excellent leaving group and they are versatile substrates for nucleophilic substitution reactions (Ding et al. 2011). The most widely used tosylating agent is the tosyl chloride (TsCl) which is more reactive than tosyl anhydride and *p*-toluenesulfonic acid. Generally, TsCl is used for the preparation of tosylates in the presence of a base (Adlington et al. 1981; Holand & Epsztein 1977; Kabalka et al. 1986). As other alcohols,

the hydroxyl group of glycerol could be changed into a tosylate. Therefore, it was an object of this study to transform the hydroxyl group of glycerol into a tosyl group where it could be used as an intermediate to produce other derivatives. Besides the possibility to act as an intermediate, the tosylates of glycerol were tested for their antibacterial properties.

MATERIALS

Glycerol (>99%) was purchased from J.T. Baker and *p*-TsCl was purchased from Fluka. All other reagents were of analytical grade and used as received unless stated otherwise.

METHODS

PREPARATION OF GLYCERYL TOSYLATES

A mixture of glycerol (5.53 g, 0.06 mol), pyridine (4 mL, excess) and *p*-toluenesulfonyl chloride (9.53 g, 0.05 mol) was stirred in 50 mL dichloromethane and the progress of the reaction was monitored by thin layer chromatography. On completion of the reaction, the reaction mixture was washed free from pyridine with 1 N hydrochloric acid, and finally water. The organic layer was separated from the aqueous layer. Sodium sulphate anhydrous then was added into the organic layer, filtered and the solvent was then evaporated to give crude tosylated product of glycerol. Based on thin layer chromatography analysis, the crude product mixture (10.5 g) was then chromatographed on a silica gel column eluting with hexane-Et_2O (v/v) = 5:5 to give three individual components (Compound 1, 2 and 3 refer to mono-, di- and tri-tosylate of glycerol, respectively) and analyzed by infrared and nucleus magnetic resonance spectroscopy besides X-ray crystallography where possible. Compound 1: ^{1}H NMR ($CDCl_3$, 600 MHz): δ 2.39 (s, 3H), 3.57 (m, 1H), 3.64 (m, 1H), 3.89 (m, 1H), 4.03 (m, 2H), 7.29 (d, J=8.4, 2H), 7.73 (d, J=8.4, 2H). ^{13}C NMR ($CDCl_3$, 600 MHz): δ 21.7, 62.8, 69.7, 70.9, 128.1, 130.1, 132.3, 145.4. Compound 2: ^{1}H NMR ($CDCl_3$, 600 MHz): δ 2.39 (s, 6H), 3.98 (m, 5H), 7.28 (d, J=8.4, 4H), 7.69 (d, J=8.4, 4H). ^{13}C NMR ($CDCl_3$, 600 MHz): δ 21.7, 67.3, 69.6, 128.0, 130.0, 132.1, 145.4. Compound 3 : ^{1}H NMR ($CDCl_3$, 400 MHz) : δ 2.49 (s, 9H), 4.08 (m, 4H), 4.69 (m, 1H), 7.36 (d, J=8.0, 6H), 7.70 (d, J=8.0, 6H). ^{13}C NMR ($CDCl_3$, 400 MHz) : δ 21.7, 66.3, 74.4, 128.0, 130.0, 131.8, 145.6. Crystal data for compound 3 : $C_{24}H_{26}O_9S_3$, T = 100 K, triclinic, a = 7.6887 (3) Å, b = 12.9635 (5) Å, c = 13.6887 (5) Å, α = 98.943 (3)° and β = 100.292 (3)°. V = 1265.91 (8) Å^3 (Yusof et al. 2012).

DISC DIFFUSION TEST METHOD

Staphylococcus aureus and *Pseudomonas aeruginosa* were grown for 24 hours on trypton soy agar. Suspensions of these tested bacteria were prepared in sterile water. The concentrations of the bacteria were adjusted to 1.0×10^6 CFU. A sterile swab was submerged in the suspensions containing bacteria and then swiped on the entire surface of the agar. Tryptone soy agar was inoculated by bacteria concentrations. 1.0, 5.0 and 10.0 mg/mL of the sample containing each of Compound 1, 2 and 3 in dimethyl sulfoxide (DMSO) were prepared. A sterile filter paper disc was dipped in the selected sample concentration and placed on the agar with sterilized forceps. Disc dipped into solvent was applied as a negative control. The agar plates (in an inverted position) containing the filter paper disc of all concentrations were incubated in an oven at 35°C for 24 h. After incubation, the diameter of each inhibition zone (in mm) was measured. Studies were performed in duplicates.

RESULTS AND DISCUSSION

Glycerol was reacted with *p*-TsCl in the presence of pyridine. A thin layer chromatography (TLC) analysis showed that the reaction mixture consists of three new components where the R_f values of mono-, di- and tri-tosylates of glycerol were 0.11, 0.48 and 0.64, respectively. After work up procedure, the concentration of mono- decreased compared to crude reaction mixture (based on TLC analysis), indicating that some of mono- was extracted into aqueous layer during washing process.

Reaction of glycerol with *p*-TsCl resulted in the formation of three components eventhough it was carried out in the excess of glycerol. The formation of di- and tri-tosylate of glycerol besides mono-tosylate of glycerol could possibly be summarized as in Figure 1.

Each component was structurally characterized where the infrared spectra (Figure 2) shows that the presence of hydroxyl group is no longer detected in tri-tosylate of glycerol indicated by the absence of broad peak at the wavenumber of 3286.27 cm^{-1} which is assigned for hydroxyl group.

The ^{1}H and ^{13}C NMR analyses confirmed that the structure of Compound 1 was mono-tosylate of glycerol, Compound 2 was di-tosylate of glycerol and compound 3 was tri-tosylate of glycerol.

Tri-tosylate of glycerol formed a single crystal from recrystallization process which was suitable for x-ray crystallography analysis (Figure 3). The crystal system of *tri*-tosylate of glycerol is triclinic wherein a = 7.6887 (3) Å, b = 12.9635 (5) Å, c = 13.6887 (5) Å, α = 98.943 (3)° and β = 100.292 (3)°.

The weak hydrogen bonds occur internally in the molecule and also externally between the molecules as shown in Figure 4. Detailed crystal structure data was published elsewhere (Yusof et al. 2012).

Mono-, di- and tri-tosylate of glycerol were tested for their antibacterial properties by using disc diffusion test method. The test was conducted according to Hung et al. (2010). Tables 1 and 2 show the inhibition zones for mono-, di- and tri-tosylate of glycerol against

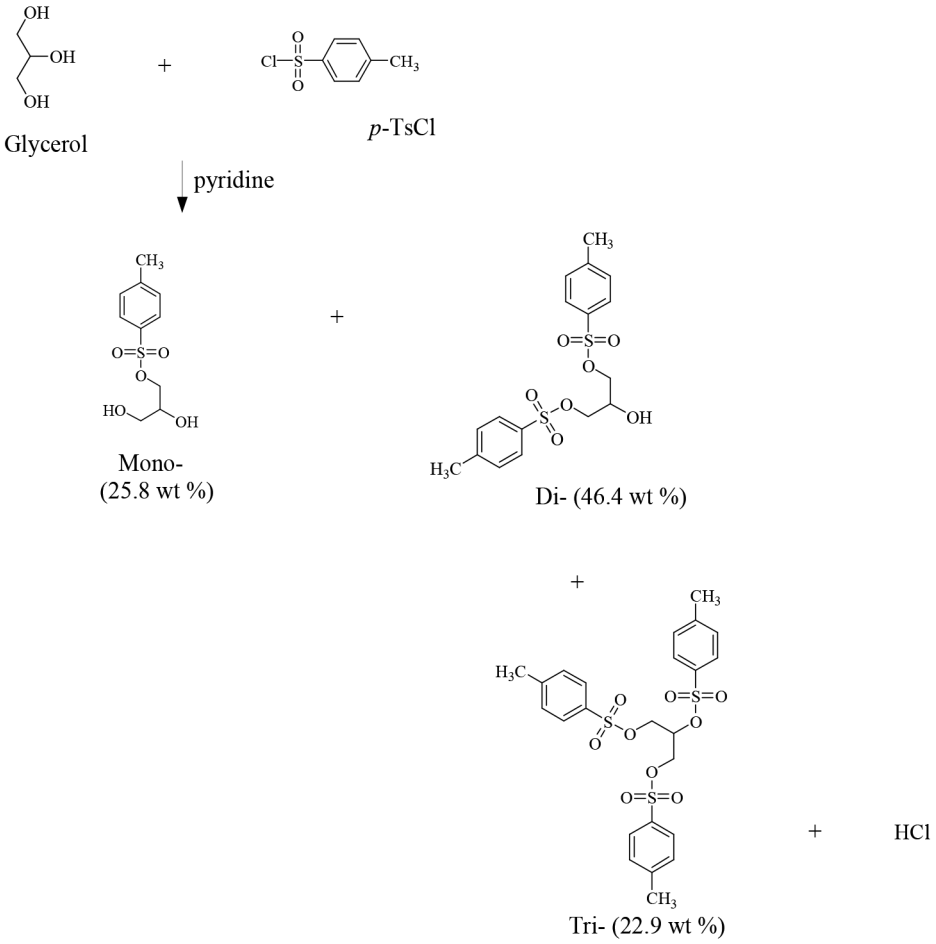
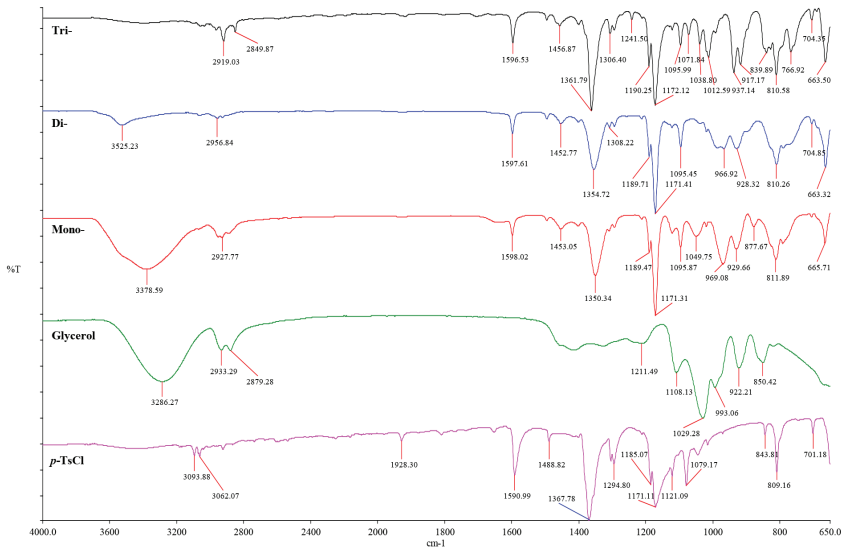


FIGURE 1. Tosylation process of glycerol with *p*-TsCl in the presence of pyridine



Note : Mono- = Mono-tosylate of glycerol
Di- = Di-tosylate of glycerol
Tri- = Tri-tosylate of glycerol
p-TsCl = *p*-toluensulfonyl chloride

FIGURE 2. Infrared spectra of mono-, di- and tri-tosylate of glycerol compared to infrared spectra of glycerol and *p*-TsCl

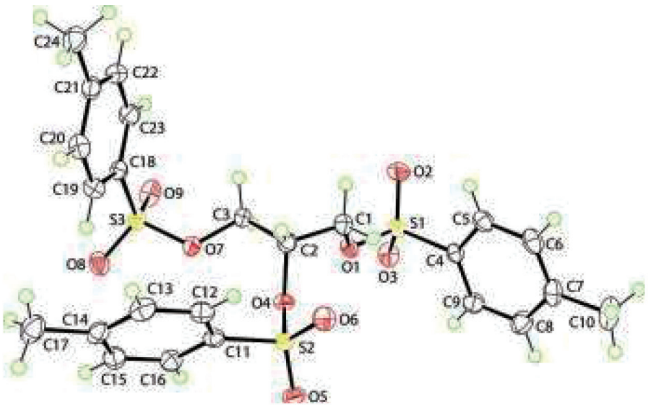


FIGURE 3. ORTEP diagram of 1,3-Bis{[(4-methylphenyl) sulfonyl] oxy}propan-2-yl 4-methylbenzenesulfonate

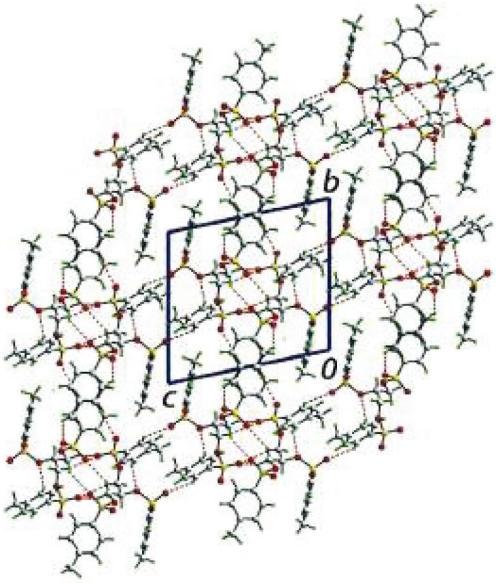


FIGURE 4. The weak C—H · · · O interactions are shown as dashed lines

TABLE 1. Antibacterial test of mono-, di-, and tri-tosylate of glycerol against *Staphylococcus aureus* (gram positive bacteria) bacteria at different concentrations

Sample	Zone of inhibition(mm)(Mean ± SD)		
	1 mg/mL	5 mg/mL	10 mg/mL
Mono-	6.5 ± 0.7	7.5 ± 0.7	7.5 ± 0.7
Di-	7.0 ± 0.1	7.0 ± 1.4	8.5 ± 0.7
Tri-	6.0 ± 0.1	6.5 ± 0.7	6.5 ± 0.7
Negative control	0	0	0

TABLE 2. Antibacterial test of mono-, di-, and tri-tosylate of glycerol against *Pseudomonas aeruginosa* (gram negative bacteria) bacteria at different concentrations

Sample	Zone of inhibition(mm)(Mean ± SD)		
	1 mg/mL	5 mg/mL	10 mg/mL
Mono-	6.5 ± 0.7	7.5 ± 0.7	9.0 ± 1.4
Di-	6.5 ± 0.7	7.5 ± 0.7	8.0 ± 0.1
Tri-	6.5 ± 0.7	7.5 ± 0.7	10.0 ± 1.4
Negative control	0	0	0

Staphylococcus aureus and *Pseudomonas aeruginosa*, respectively.

Mono-, di- and tri-tosylate of glycerol showed antibacterial activity at the concentration as low as 1 mg/mL against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, respectively, in the range of the experimental study.

Conclusion

The reaction of glycerol with *p*-TsCl has led to the formation of tri- and di- besides mono-tosylate of glycerol eventhough the reaction was done at the ratio of 1.2:1.0 of glycerol to *p*-TsCl. These three compounds of glyceryl tosylates were successfully separated, characterized and exhibited antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

The preliminary study of antibacterial activity of glyceryl tosylates may indicate its potential application in possible chemical industry.

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