http://dx.doi.org/10.4314/jpb.v12i1.8
Vol. 12 no. 1, pp. 54-59 (March 2015)
http://ajol.info/index.php/jpb

Journal of
PHARMACY AND BIORESOURCES

Synthesis and antinociceptive activity of methyl nicotinate

Osayemwenre Erharuyi[1], Ighodaro Igbe[2*], Abiodun Falodun[1], Rosemary Enadeghe[3] and Osamede Igbinedion[1]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy;
[2]Department of Pharmacology and Toxicology, Faculty of Pharmacy;
[3]Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Nigeria

Received 31st March 2015; Accepted 31st March 2015

Abstract
Methyl nicotinate (methyl-3-pyridinecarboxylate) is a methyl ester of nicotinic acid – a type of B vitamin called niacin. It was prepared by esterification of nicotinic acid by refluxing with methanol in the presence of concentrated sulphuric acid, esterification product obtained was extracted into organic solvent (chloroform) after neutralization of the reaction mixture with 10% sodium bicarbonate. The product was purified by column chromatography and purity ascertained by thin layer chromatography. Structure of the desired product was confirmed by NMR and Mass spectroscopy. Methyl nicotinate was obtained as a white powder (m.p. 40-42^{o}C, 23.39% yield). The product was evaluated for its antinociceptive activity using the acetic acid-induced writhing and the hot plate test in mice. The synthesized compound exhibited effective peripheral and central antinociceptive activity. Oral doses of methyl nicotinate (5 and 10 mg/kg) caused a significant reduction in the number of writhes induced by acetic acid ($P < 0.05$) and prolonged the reaction latency to thermally-induced pain in mice.

Keywords: Methyl nicotinate, esterification, antinociceptive activity

INTRODUCTION

Nociception is a sensory activity induced by a noxious stimulus, while pain is a sensation that contains other components in addition to nociception. The experience of pain has a distinctly unpleasant character which is associated with actual or potential tissue damage (Merskey, 1979). Pain is a multidimensional experience that is essential for the maintenance and preservation of an individual. Under normal circumstances, primary afferent pain fibres activate particular central pathways that engage protective mechanisms at several functional levels: autonomic, homeostatic, motoric, behavioural and mnemonic (Kurlekar and Bhatt, 2004). However, injury or disease can alter the balance of this system and result in persistent, pathological pain. Analgesic substances, such as the NSAIDs and opioids that interact with the transmitters and modulators of the pain system are helpful but never sufficient in the management of pain (Hewitt *et al*., 2009). For this reason, there is a continued need for the development of more analgesics for the alleviation and control of both acute (immediate) and chronic (long-term, pathological) pain.

Methyl Nicotinate also known as methyl-3-pyridinecarboxylate, methylpyridine

* Corresponding author. *E-mail*: igbe.ighodaro@uniben.edu *Tel*: +234 (0) 8166058559
ISSN 0189-8442

-3-carboxylate and nicotinic acid methyl ester is an ester that is derived from nicotinic acid. Niacin – a water-soluble B vitamin –vitamin B3 - is the term used to describe two related compounds, Nicotinic acid and nicotinamide (Müller *et al*., 2003; Boatman *et al*., 2008). Presently, methyl nicotinate was synthesized by simple esterification reaction, comprising reacting nicotinic acid with methanol in the presence of sulphuric acid catalyst. Esterification reaction is usually a slow process and requires high temperature, high vacuum, and an esterification catalyst such as sulphuric acid, p-toluene sulfonic acid or tosic acid to affect the reaction rate (Qiao *et al*., 2006; Liu *et al*., 2006; Cai *et al*., 2007; Schmitt *et al*., 2008). The reaction can often be performed with little or no solvent or in the presence of a solvent such as benzene. This is a relatively low cost approach, but requires thermally stable reagents and products. The resulting mixture may be warmed to reflux and then allowed to cool after the reaction has been effected. In general, this is an equilibrium reaction in that appreciable quantities of both alcohol and ester are present under equilibrium condition. The equilibrium may be influenced by either removing one product from the reaction mixture (for example, removal of the water by azeotropic distillation or absorption by molecular sieves) or by employing an excess of one reactant (Hangx *et al*., 2001; Ismail *et al*., 2001; Tang *et al*., 2003; Maris and Jurgen, 2006). The reaction product, which is insoluble in water, may be subjected to one or more washes e.g. in an alkaline wash such sodium bicarbonate or ammonia for neutralization purposes, and if desired, a pure crystalline product may be obtained by removal of solvent, if present, and subsequent recrystallization from a suitable solvent such as methanol, or a liquid hydrocarbon like heptane. The final product is obtained as a precipitate, which is collected by filtration, washed with solvent and dried in vacuum to remove any residual solvent. Esters can also be formed by various other reactions. These include the reaction of an alcohol with an acid chloride (R-CO-Cl) or an anhydride (R-CO-O-COR'). Early studies into the chemical mechanism of esterification, concluded that the ester product (R-CO-OR') is the union of the acyl group (R-C=O-) from the acid, *RCO*-OH, with the alkoxide group (R'O-) from the alcohol, R'-OH rather than other possible combinations (Zhang *et al*., 1995; Rönnback *et al*., 1997; Liu *et al*., 2006; Otera and Nishikido, 2009).

In an attempt to find more options and possibly more tolerable agents for pain management, methyl nicotinate herein was investigated for potential analgesic activity.

EXPERIMENTAL

General synthetic procedure. A mixture of pure nicotinic acid (10.0g, 0.08 mol.), 30 mL of absolute methanol and 1.0 mL of concentrated H_2SO_4 were refluxed for 13 hours on a steam bath. The resulting solution was cooled and transferred on to a 20.0g of crushed ice. Sodium bicarbonate (4.0 mL) was added to render the solution strongly alkaline. The mixture was extracted with 20.0 mL portions of chloroform four times and the residue distilled under reduced pressure. The reaction was monitored with Thin-layer chromatography (TLC) at intervals. The synthesized compound was purified by column chromatography and product confirmed by NMR and mass spectra data.

Animals. All experiments were performed using Swiss albino mice (22- 30g) of either sex. All the animals were obtained from the laboratory animal house of the Faculty of Pharmacy, University of Benin. The animals were fed with standard pellitized Vital Feed Growers (Grand Cereals LTD, Benin City) and water *ad libitum*. Animals were exposed to natural lighting conditions and were handled in accordance with the international

principles guiding the use and handling of experimental animals.

Test for antinociceptive activity

Acetic acid-induced writhing in mice. The analgesic effect of extract was evaluated by the acetic acid-induced mouse writhing test (Koster *et al*., 1959). Swiss albino mice (22-30g) were divided into four groups of five animals per group. Animals in group one were administered 5 mL/kg of distilled water orally to serve as a negative control. Animals in group two and three were administered 5.0 mg/kg and 10.0 mg/kg methyl nicotinate respectively while group four animals were given 100 mg/kg of Aspirin as a positive control. All drugs were administered orally. Acetic acid (10 mL/kg of 0.6%v/v) was administered intraperitoneally after one hour of administration of the drug. The number of writhes by each mouse was counted immediately after acetic acid administration at intervals of 5 minutes for a period of 30 minutes.

Hot plate test. The experimental method of Eddy and Leimbach (1953) was used with slight modification. Swiss albino mice (22-30g) were randomly divided into four groups of five animals per group. The animals were individually placed on a hot plate maintained at a constant temperature of 55 ± 1˚C, the time interval from placement and shaking/licking of the paw or jumping was recorded as an index of response latency. The initial reaction time of each animal was determined and the cut-off time was set at 30 seconds. Group one was treated orally with distilled water 5.0 mL/kg (negative control). Animals in group two and three were orally administered 10.0 mg/kg and 5.0 mg/kg methyl nicotinate respectively while group four animals were given 2 mg/kg of morphine subcutaneously, as a standard. The animals were placed on the hot plate at 0, 30, 60, 90 and 120 minutes after treatment and the time taken for either paw licking or jumping was recorded.

Statistical analysis. Data were expressed as the mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test using Graph pad prism version 6.0. $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Synthesis of methyl nicotinate. Methyl nicotinate have been synthesized from nicotinic acid via sulphuric acid catalyzed esterification reaction. The reaction of nicotinic acid occurred in the presence of concentrated sulphuric acid solution under refluxing methanol for 13 hours, to obtain methyl nicotinate, which underwent further purification by silica gel column chromatography in a solvent system of petroleum ether/ethyl acetate (4:1). Purity of the synthesized compound was ascertained by TLC. Analysis of the ^{1}HNMR and Mass spectral data confirms the desired product.

Methyl nicotinate (3-carboxymethylpyridine): White powder, yield 23.39%. m.p. = 40-42^{o}C.
IR (KBr cm^{-1}): 1728.1(C=O), 1587.3(C=N-), 1429.2(C=C, aromatic), 1292.2-1120.6(C-O-C).
^{1}HNMR (δ_H): 9.34 (s, H-2), 8.97 (br d, J = 8.0, H-4, H-6), 8.04 (br s, H-5), 4.05 (s, O$\underline{CH_3}$).
EIMS (m/z): 137.0 [M^+], 122.0 [$M-CH_3$]$^+$, 106.0 [$M-OCH_3$]$^+$.

The interaction between a carboxylic acid and an alcohol is a reversible process and proceeds very slowly (Qiao *et al*., 2006; Cai *et al*., 2007; Xu *et al*., 2011). Equilibrium is only attained after refluxing for several days. If, however, about 3 per cent (of the weight of the alcohol) of either concentrated sulphuric acid or of dry hydrogen chloride is added to the mixture, the same point of equilibrium can be reached after a few hours (Furniss *et al*., 1989). Herein, we have used 1 mL of concentrated sulfuric acid representing 3% of the weight of methanol used (30 mL). It is possible however to use more of the sulphuric acid catalyst to increase the reaction rate and

subsequently the reaction time is reduced further, but this option have been shown to yield more reaction by-products. Hence the least amount as possible of the acid catalyst was employed so as to limit the production of by products and the product (methyl nicotinate) can be obtained in a relative pure form in a one-pot synthetic procedure. On the other hand, when equimolecular quantities of the acid and alcohol are employed, only about two-thirds of the theoretically possible yield of ester is obtained. According to the law of mass action, the equilibrium may be displaced in favour of the ester by the use of an excess of one of the components. In this case an excess of the alcohol was used. This method of esterification, in general, gives good yields with primary alcohols and fairly good yields with secondary alcohols. The method is unsatisfactory for use with tertiary alcohols owing to competing alkene formation from an acid catalyzed dehydration (Furniss *et al*., 1989).

Table 1: Anti-nociceptive effect of methyl nicotinate in acetic acid-induced writhing in mice

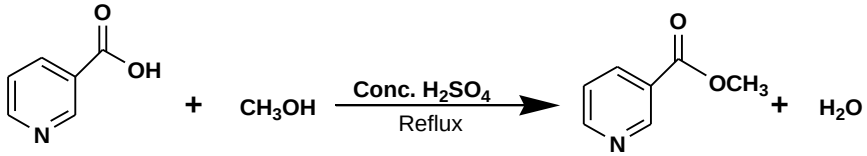
Group/Treatment	Number of Writhings (Mean ± SEM)					
	0 – 5 min	5–10 min	10–15 min	15–20 min	20–25 min	25–30 min
Negative control (5 mL/kg)	15.8±3.72	29.0±5.50	40.0±6.76	49.8±8.50	57.6±9.90	64.20±11.35
Methyl nicotinate (5 mg/kg)	11.00±2.62	22.60±5.26	28.80±6.35[a]	37.00±8.00	42.2±8.46	46.20±9.37*
Methyl nicotinate (10 mg/kg)	8.40±2.95	18.2±8.07	23.2±8.55*	30.20±10.92*	36.00±12.50*	40.6±13.20*
Aspirin (100 mg/kg)	6.00±1.87	19.25±2.17	26.00±2.48*	32.25±3.12*	37.75±4.21*	42.04±4.30*

Data represents Mean ± Standard Error of Mean (SEM), n = 5. *P < 0.05 compared to negative control.

Table 2: Anti-nociceptive effect of methyl nicotinate in the hot plate test:

Group/Treatment	Reaction time in seconds (Mean ± SEM)				
	0 min	30 min	60 min	90 min	120 min
Negative control (5mL/kg)	5.77 ± 0.43	5.80 ± 0.61	4.95 ± 0.66	6.45 ± 0.29	6.12 ± 0.43
Methyl nicotinate (5 mg/kg)	4.56 ± 0.40	9.64 ± 0.87*[a]	13.30 ± 0.62*	16.64 ± 1.12*	13.62 ± 0.68*[a]
Methyl nicotinate (10 mg/kg)	4.26 ± 0.73	8.86 ± 1.84*[a]	14.46 ± 1.19*	14.62 ± 1.05*	15.62 ± 1.08*[a]
Morphine(2 mg/kg)	6.02 ± 0.41	13.66 ± 1.46*	15.32 ± 1.08*	16.64 ± 0.98*	16.12 ± 0.43*

Data represents Mean ± Standard Error of Mean (SEM), n = 5. *P < 0.05 compared to negative control, [a]P < 0.05 compared to morphine.



Scheme 1: Sulphuric acid catalysed esterification of nicotinic acid and methanol.

Antinociceptive activity of 3-carboxymethyl pyridine (methyl nicotinate) was evaluated using the acetic acid-induced writhing reflex and the hot plate-induced paw edema representing the commonly used methods for antinociceptive activity for peripheral and central pain model respectively. Table 1 shows the effect of the methyl nicotinate on acetic acid induced mouse writhing. Methyl nicotinate (5 and 10 mg/kg) produced a decrease in the number of writhes compared to distilled water (negative control). At 5

mg/kg methyl nicotinate, there was a significant difference at the 30th minute, while at a higher dose of 10 mg/kg, significant difference was observed from the 10th through the 30th minute ($P < 0.05$). At the dose of 10 mg/kg, the analgesic effect of methyl nicotinate was comparable to that of aspirin throughout the 30 minutes time period ($P > 0.05$). The acetic acid-induced writhing in mice is widely used for the evaluation of peripheral antinociceptive activity (Gene *et al*., 1998). It causes inflammation via increase in capillary permeability and release of endogenous pain mediators (Amico-Roxus *et al*., 1984; Raj, 1996). It is very sensitive and able to detect antinociceptive effect of compounds at dose levels that may appear inactive in other methods. As shown in the result (table 1), oral administration of methyl nicotinate caused a significant reduction ($P < 0.05$) in the number of writhes induced by acetic acid at the two doses (5 and 10 mg/kg), and this was sustained throughout the 30 minutes period, suggesting that analgesic effect of the drug may be peripherally mediated. The drug methyl nicotinate have a long duration of action and faster onset of action peripherally.

For the hot plate test, methyl nicotinate was able to cause a significant increase in reaction time after 30 minutes of administration compared to normal saline ($P < 0.05$). The reaction time was not different between the two doses tested (5 and 10 mg/kg). In comparison to morphine (standard), no significant difference was found in the reaction time at both 5 and 10 mg/kg from the 60th minute. The observed increase in reaction time for the test group was sustained over the 120-minute observation period. The hot plate method is one of the most common tests for centrally mediated nociception based on a phasic stimulus of high intensity and is also used to measure the latencies of pain response (Chapman *et al*., 1985; Wigdor *et al*., 1987; Mandegary *et al*., 2004). Pain induced by thermal stimulus of the hot plate is specific for centrally mediated nociception (Heidary *et al*., 2009). The ability of methyl nicotinate to prolong the reaction latency to thermally-induced pain in mice could further suggest a central analgesic effect. The effect however is slow in onset of action but with long duration of action. Therefore, methyl nicotinate could posses both peripheral and central analgesic effect and this might be said to be the first report of the occurrence of this activity in 3-carbomethoxypyridine.

Conclusion. In the present study, methyl nicotinate have been synthesized from nicotinic acid by esterification reaction. The result of the analgesic activity indicated that oral doses of methyl nicotinate could produce effective antinociceptive/ analgesic activity peripherally and centrally.

Acknowledgement

The authors thank the Departments of Pharmacology, and Pharmaceutical Chemistry, University of Benin for their facility. The STEP-B Award (HME/STEP-B/IOT/33/Vol.1/6) of the Federal Ministry of Education, Nigeria is also appreciated.

REFERENCES

Amico-Roxus M, Caruso A, Trombadore S, Scifo R, Scapagini U. (1984). Gangliosides antinociceptive effect in rodents. *Arch Int Pharmacodyn Ther* 272:117-124.

Bentley GA, Newton SH, Starr J. (1981). Evidence for the action of morphine and enkephalins and sensory nerve endings in the mouse peritoneum. *Br. J. Pharmacol*., 73: 325-332.

Boatman PD, Richman JG, Semple G. (2008). Nicotinic acid receptor agonists. *J. Med. Chem*. 51:7653.

Cai Y, Huang D, Wan H, Guan, G. (2007). Preparation of a silica gel confined ionic liquid and its use as catalyst for esterification. *Fine Chemicals* 24:1196-1199.

Chapman CR, Casey KL, Dubner RK, Foley M, Gracely RH, Reading AE. (1985) "Pain measurement: an overview," *Pain* 22(1):1-31.

Eddy NB, Leimbach D. (1953). Synthetic analgesics. II diethienylbutenyl- and dithienylbutylamines. *J. Pharmacol. Exp. Ther.* **107.** 385-393.

Furniss BS, Hannaford AJ, Smith PWG, Tatchell AR. (1989). Carbohylic acid derivatives. In: Vogel's Textbook of Practical Organic Chemistry (5th ed.), Longman Sci & Tech-John Wiley & Sons Inc., NY; pp 695-696.

Gene RM, Segura L, Adzet T, Marin E, Inglesias J. (1998). Heterotheca inuloides: anti-inflammatory and analgesic effects. *J. Ethnopharmacol*., 60:157-162.

Hangx G, Kwant G, Maessen H, Markusse P, Urseanu I, (2001) "Reaction Kinetics of the esterification acid towards ethyl acetate", Technical report on the European Commission (INTINT) Deliverable 22.

Heidari MR, Foroumadi A, Noroozi H, Samzadeh-Kermani A, Azimzadeh BS. (2009) Study of the anti-inflammatory and analgesic effects of novel rigid benzofuran-3, 4-dihydroxychalcone by formalin, hot plate and carrageenan tests in mice. *Pak. J. Pharm. Sci*., 22(4):395-401.

Hewitt DJ, Hargreaves RJ, Curtis SP, Michelson D. (2009). "Challenges in analgesic drug development," *Clinical Pharmacology &Therapeutics* 86(4):447-450.

Ismail K, Baris B, Umur D. (2001). Esterification of acetic acid with ethanol catalyzed by an acidic ion-exchange resin", *Turk J Engin Environment Sci* 25:569-577.

Koster R, Anderson M, De-Beer EJ. (1959). Acetic acid for analgesic screening. *Federation Proceedings*, 18: 412-418

Kurlekar PN, Bhatt JD. (2004). Study of the antinociceptive activity of Fluoxetine and its interaction with morphine and Naloxone in mice. *Indian J Pharmacol* 36:369-372.

Liu Y, Lotero E, Goodwin J. (2006). A comparison of the esterification of acetic acid with methanol using heterogeneous versus homogeneous acid catalysis. *Journal of Catalysis* 242:278-286.

Mandegary A, Sayyah M, Heidari MR. (2004). Antinociceptive and anti-inflammatory of the seed and root extract of *Ferula gummosa* Boiss in mice and rats. *DARU*, 12(2):58-62.

Maris TS, Jurgen G. (2006). Esterification of acetic acid with isopropanol coupled with pervaporation part 1 (Kinetics and pervaporation studies), *Chem. Eng. Journal* 123:1-8.

Merskey H. (1979). Pain terms: a list with definitions and notes on usage. Recommended by the lASP Subcommittee on Taxonomy. *Pain* 6:249–252.

Müller B, Kasper M, Surber C, Imanidis G. (2003). Permeation, metabolism and site of action concentration of nicotinic acid derivatives in human skin. Correlation with topical pharmacological effect. *Eur. J. Pharm. Sci*. 20(2):181-95.

Otera J, Nishikido J. (2009). Reaction of Alcohols with Carboxylic Acids and their Derivatives In: Esterification: methods, reactions, and applications (2nd). Vch Publisher; pp 3-157.

Qiao K, Hagiwara H, Yokoyama C. (2006). Acidic ionic liquid modified silica gel as novel solid catalysts for esterification and nitration reactions. *J. Mol. Catal. A: Chem*. 246:65-69.

Raj PP (1996). Pain mechanism. In: Pain medicine: A comprehensive review (1st ed.), Mosby-year book, Missouri, pp 23.

Ronnback R, Salmi T, Vuori A, Haario H, Lehtonen J, Sundqvist A, Tirronen E. (1997). Development of a kinetic model for the esterification of acetic acid with methanol in the presence of a homogeneous acid catalyst. *Chem. Eng. Sci* 52:3369-3381.

Schmitt M, Blagov S, Hasse H. (2008) Mastering the reaction is the key to successful design of heterogeneously catalyzed reactive distillation: A comprehensive case study of hexyl acetate synthesis. *Ind. Eng. Chem. Res*. 47:6014-6024.

Tang, YT, Huang HP, Chain IL. (2003). Design of a complete EtOAC reactive distillation system, *J. Chem. Eng. Japan* 36:1352-1363.

Wigdor S, Wilcox GL. (1987). Central and systemic morphine-induced anti-nociception in mice: contribution of descending serotonergic and noradrenergic pathways, *JPET* 242(1):90-95.

Xu J, Zhang J, Yin X, Yang D, Zhang H, Qian J, Liu L, Liu X. (2011). Esterification process to synthesize Isopropyl chloroacetate catalyzed by Lanthanum dodecyl sulfate. *Bra. J Chem. Eng.* 28(02):259-264.

Zhang HL, Feng GY, Wei TJ. (1995). New development and kinetics of esterification Henan *Chemical Industry* 2:5-8.