Journal of Advances in Science and Technology

Vol. IX, Issue No. XIX, May-2015, ISSN 2230-9659

REVIEW ARTICLE

A STUDY ON THE SYNTHESIS OF TETRAPEPTIDES

AN INTERNATIONALLY INDEXED PEER REVIEWED & REFEREED JOURNAL

www.ignited.in

Journal of Advances in Science and Technology
Vol. IX, Issue No. XIX, May-2015, ISSN 2230-9659

A Study on the Synthesis of Tetrapeptides

Pradeep Singh[1] Dr. Dev Sharan Singh[2]

[1]Research Scholar – NWMDIU, SA

[2]Dexo Chem Laboratories, Ankleshwar

----------♦----------

INTRODUCTION

1,4-Butanediol is the natural compound with the formula $HOCH_2CH_2CH_2CH_2OH$. This colorless sticky fluid is derived from butane by appointment of alcohol groups at each end of the chain. It is amongst the four established isomers of butanediol. In its manufacturing synthesis, acetylene reacts with two equivalents of formaldehyde to shape 1,4-butynediol. This type of acetylene-based procedure is descriptive of what is identified as "Reppe chemistry", after German chemist Walter Reppe. Hydrogenation of 1,4-butynediol gives 1,4-butanediol.

First, propylene oxide is changed to allyl alcohol. The allyl alcohol is then hydro-formylated to 4-hydroxybutyraldehyde. Hydrogenation of this aldehyde yields 1,4-butanediol.

It is also manufactured on an manufacturing scale from maleic anhydride in the Davy process, which is first changed to the methyl maleate ester, then hydrogenated. Other alternatives are from butadiene, allyl acetate and succinic acid.

A genetic path to BDO has been commerciallized that uses a hereditarily customized organism. The bio-synthesis proceeds via 4-hydroxybutyrate.

1,4-Butanediol is used scientifically as a solvent and in the manufacture of several types of plastics, elastic fibers and polyurethanes. In organic chemistry, 1,4-butanediol is used for the synthesis of γ-butyrolactone (GBL). In the company of phosphoric acid and high temperature, it dehydrates to the important solvent tetra-hydrofuran. At about 200 °C in the presence of soluble rutheniumcatalysts, the diol undergoes dehydrogenation to shape butyrolactone.

World production of 1,4-butanediol was claimed to be about one million metric tons per year and advertise cost is about 2,000 USD (1,600 EUR) per ton (2005). In 2013, worldwide production was claimed to be billions of lbs.

Almost half of it is dehydrated to tetra-hydrofuran to compose fibers like Spandex. The major producer is BASF.

It is also used as a leisure drug known by various users as "One Comma Four", "One Four Bee" or "One Four B-D-O". It exerts possessions similar to γ-hydroxybutyrate (GHB), which is a metabolic product of 1,4-butanediol. Misuse has also resulted in addiction and death.

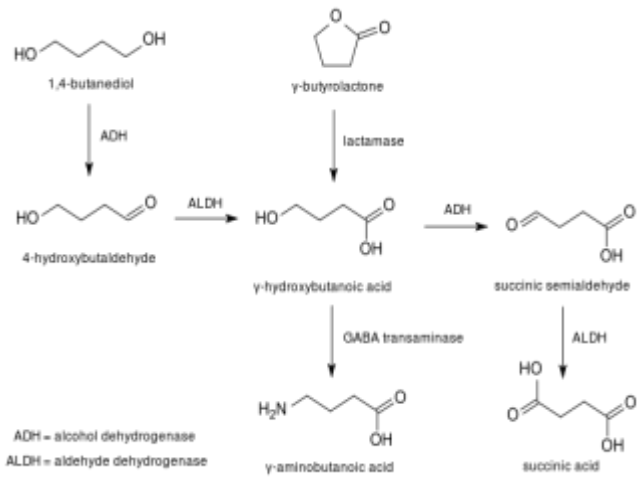
1,4-Butanediol is transformed to GHB by the enzymes alcohol dehydrogenase and aldehyde dehydrogenase and conflicting levels of these enzymes may report for differences in effects and side effects between users. While co-administration of ethanol and GHB already poses severe risks including death, co-administration of ethanol with 1,4-butanediol is probable to have even more potentially serious risks. This is because the same enzymes are accountable for metabolizing alcohol so there is a tough possibility of an unsafe drug interface. Emergency room patients who overdose on both alcohol and 1,4-butanediol frequently present with symptoms of ethanol intoxication at first and as the ethanol is metabolized the 1,4-butanediol is then capable to healthier struggle for the enzyme and a second period of intoxication ensues as the 1,4-butanediol is transformed to GHB.

1,4-Butanediol was designated by the National Cancer Institute and chosen for assessment by the NTP because of elevated fabrication volume, the prospective for worker revelation, the requirement of sufficient toxicological characterization and the lack of assessment for carcinogenic potential. As acknowledged in the scientific writing, 1,4-butanediol is quickly captivated and metabolized to –hydroxybutyric acid in animals and humans. A metabolism and disposition research conducted in F344/N rates by the NTP confirmed the quick and wide-spread translation of 1-[^{14}C]-1,4-butanediol to ^{14}CO. Because of this quick and widespread alteration, the toxicological profile of 1,4-butanediol reflects that of hydroxyl-butyric acid. –Hydroxy-butyric acid is a unsurprisingly occurring chemical originated

in the brain and tangential tissues which is transformed to succinate and processed through the tri-carboxylic acid cycle. Although the purpose of -hydroxybutyric acid in peripheral tissues is unknown, in the brain and neuronal tissue it is thought to work as a neuro-modulator. Hydroxybutyric acid enthusiastically crosses the blood-brain barrier and oral, intra-peritoneal or intravenous organization elicits characteristic neuro-pharmacologic responses. These same responses are observed after administration of 1,4-butanediol.

The lactone of -hydroxybutyric acid, 1-butyrolactone, is also quickly transformed to -hydroxybutyric acid by enzymes in the blood and liver of animals and humans. 1-Butyrolactone was before measured by the NTP in 14-day and 13-week toxicology studies and 2-year toxicology and carcinogenesis studies in F344/N rates and B6C3F1 mice. No organ-specific toxicity found in the toxicology studies. In the carcinogenesis research, an equivocal response found in male mice, based on a minor rise in the occurrence of pheo-chromocytomas of the renal medulla. Because of the quick and wide alteration of -butyrolactone to -hydroxybutyric acid, the assessment of -butyrolactone was in fact an assessment of - hydroxybutyric acid.

This description presents a review of the present literature which documents that both 1,4-butanediol and -butyrolactone are quickly metabolized to -hydroxybutyric acid, and the pharmacologic and toxicologic reacts to these chemicals are due to their metabolic alteration to -hydroxybutyric acid. Because the toxicity and carcinogenicity of -hydroxybutyric acid was completely evaluated in the NTP research of -butyrolactone, and a lack of organ-specific toxicity or carcinogenic potential was verified, it is fulfilled that there is a elevated likelihood that 1,4-butanediol would be negative in a similar set of researches. For these reasons, it is the estimation of the NTP that 1,4-butanediol should be measured not carcinogenic in animals and no further assessment of 1,4-butanediol is needed at this time.



Metabolic pathway of 1,4-butanediol, γ-butyrolactone and γ-hydroxybutyric acid (GHB).

1,4-Butanediol likely to have two types of pharmacological activities. The main psychoactive effects of 1,4-butanediol are because it is metabolized into GHB; however there is a research suggesting that 1,4-butanediol may have possible alcohol-like pharmacological effects that are not due to this transformation. One should observe that the research arrived at this end based on the judgment that 1,4-butanediol co-administered with ethanol led to potentiating of a few of the behavioral effects of ethanol. However, potentiation of ethanol's effects may merely be caused by struggle for the alcohol dehydrogenase and acetaldehyde dehydrogenase enzymes with co-administered 1,4-butanediol. The common metabolic rate-limiting steps thus brings to slowed metabolism and authorization for both compounds including ethanol's known toxic metabolite acetaldehyde.

Another research found no effect following intra-cerebroventricular injection in rats of 1,4-butanediol. This contradicts the theory that 1,4-butanediol having intrinsic alcohol-like pharmacological effects.

Like GHB, 1,4-butanediol is only secure in little amounts. Difficult effects in superior doses include, nausea, vomiting, dizziness, sedation, vertigo, and potentially death if ingested in huge amounts. Anxiolytic effects are diminished and side effects augmented when used in mixture with alcohol.

While 1,4-butanediol is not at present planned federally in the United States, a number of states have classified 1,4-butanediol as a controlled substance. In addition, individuals have been prosecuted for 1,4-butanediol under the Federal Analog Act as considerably comparable to GHB. A central case in New York in 2002 ruled that 1,4-butanediol could not be measured an analog of GHB under federal law, but that choice was afterward upturned by the Second Circuit. In the United Kingdom, 1,4-butanediol was listed in December 2009 as a Class C controlled substance.

A tetrapeptide is a peptide comprises of four amino acids connected by peptide bonds. Many tetra-peptides are pharmacologically active, regularly screening resemblance and specificity for a range of receptors in protein-protein signaling. Present in environment are both linear and cyclic tetra-peptides; tetra-peptides may be cyclized by a fourth peptide bond or other covalent bonds.

RESEARCH METHODOLOGY:

Materials and Methods

Polyethylenimine (PEI, branched, MW 750 kDa and 25 kDa), polyethylene glycol (PEG, MW 8 kDa and 4 kDa), 4-imidazoleacetic acid hydrochloride, lauric

acid, 1,4-butanediol diglycidyl ether (bisepoxide), acetaldehyde, propionaldehyde, butyraldehyde, hexane-1,6-diol, adipic acid, 11- mercaptoundecanoic acid, tetrachloroauric acid ($HAuCl_4$), sodium cyanoborohydride ($NaCNBH_3$), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC), 3-(3-dimethylthiazole-2- yl)-2,5-diphenyltetrazolium bromide (MTT), agarose, Tris-HCl, HEPES, ethidium bromide (EtBr), xylene cyanol, bromophenol blue and high retention dialysis tubing (cut off 12 kDa) were obtained from Sigma-Aldrich Chemical Co., USA. Specialized chemicals/reagents were purchased from their respective suppliers, viz., Bradford reagent (Bio-Rad Inc., USA), transfection reagents such as Superfect™(Qiagen, France), Fugene™(Roche Applied Science, USA), and GenePORTER™ and GenePORTER2™ (Gelantis, USA), plasmid purification kit (Qiagen, France), the plasmid pEGFPN3 (Clontech, USA) and stannous chloride (extrapure Ph Eur., BP grade, Merck, Germany). Sodium pertechnetate eluted fresh from ^{99m}Mo by solvent extraction method (Garg, M. et al., 2008) was procured from Regional Centre for Radiopharmaceuticals (Department of Atomic Energy, India). Balb C mice (2-3 month-old, 25-30g wt) were used for *in vivo* studies. All other reagents and chemicals used in the present work were procured locally and were of sufficient purity. In all experiments, deionised and Millipore-filtered (0.22µm) water was used.

Cell culture

COS-1 (Simian virus 40 transformed kidney cells of an African green monkey), HEK293 (Human embryonic kidney), HeLa (Human cervical adenocarcinoma), A549 (Human lung adenocarcinoma epithelial cell line), N2a (mouse neuroblastoma cell line) and HepG2 (Human hepatocellular carcinoma) were obtained from the cell repository facility of National Centre for Cell Sciences (NCCS), Pune, India. Cell cultures were maintained (37^{o}C, 5%CO_2–air) in Dulbecco's Modified Eagle's Culture Medium (DMEM) (Sigma, USA) with 10% heat inactivated fetal bovine serum (GIBCO-BRL-Life Technologies, UK) and 1% antibiotic cocktail of streptomycin and penicillin.

Purification of solvents

(i) Toluene: Analytical grade toluene was purified and dried by continuous refluxing over sodium metal for 4-5h and collecting the distilled solvent after discarding the initial fraction of 50ml. The dried solvent was then stored over 4^{o}A type molecular sieves.

(ii) Ethylene dichloride (EDC): AR grade solvent was refluxed over calcium hydride (2g/l) for 16h, distilled and stored under argon over molecular sieves type 4^{o}A (10g/l).

(iii) Triethylamine (TEA): AR grade TEA was refluxed with ninhydrin for 4h before distillation. The distilled TEA was again refluxed over sodium hydroxide for 4-5h followed by distillation. The dried reagent was stored over potassium hydroxide in an amber coloured reagent bottle.

(iv) N,N-Dimethylformamide (DMF): Analytical grade solvent was purified by azeotropic distillation with benzene to remove water, followed by distillation under reduced pressure. The residual solvent was shaken with activated alumina (Grade I). The alumina was removed by filtration and the filtered solvent was stored in an amber coloured bottle.

Instruments

The following instruments were used for carrying out the proposed studies.

(i) Sonicator: Nano-particles were sonicated using a Misonix 3000 sonicator, West Chester PA, USA, with total sonication time of 3min (6x30s), each sonication pulse was followed by a 30s stop time at 4^{o}C (ice-bath) with power set at 3W using a micro tip probe.

(ii) ^{1}H NMR: Spectra were recorded on a Bruker DRX-300 (300MHz FT NMR) spectrometer using appropriate solvent.

(iii) FTIR: Spectra of nano-particles were recorded on a single beam Perkin Elmer (Spectrum BX Series), USA, with the following scan parameters: scan range, 4400-400 cm^{-1}; number of scans, 16; resolution, 4.0cm^{-1}; interval, 1.0cm^{-1}; unit, %T.

(iv) Fluorescent Microscope: GFP protein expression was observed under a Nikon Eclipse TE 2000-U inverted microscope, Kanagawa, Japan, fitted with a C-FI epifluorescence filter block B-2A consisting of excitation filter Ex 450-490nm, Dichroic mirror DM 505 and barrier filter BA 520.

(v) Fluorospectrometer: Green fluorescent protein (GFP) in the soup of lysate of transfected cells was analyzed (excitation at 488nm, emission at 509nm) on NanoDrop™ ND-3300 Fluorospectrometer (U.S.A).

(vi) Confocal Microscope: In order to elucidate the path of nanoparticles inside the cells *in vitro*, these cells were exposed to fluoresceinylated nanoparticles. Differential interference contrast (DIC) and fluorescence images were acquired with a Zeiss 510 Meta confocal microscope.

Characterization of nano-particles

Zeta potential is a function of the surface charge of the particle, any adsorbed layer at the interface, as

well as the nature and composition of the surrounding medium in which the particle is suspended. It reflects the effective charge on the particles and determines **a variety of** properties such as the **interface** with DNA, with the cell surface proteo-glycans, etc.

In the current study, Zetasizer Nano ZS (Malvern Instruments, UK) was used for measuring surface charge on the particles. Samples (nanoparticles as well as nanoplexes) were **thinned** with MilliQ (deionized) water and zeta potential measurements were carried out in automatic mode and the values were presented as the average value of 30 runs. The Smoluchowski approximation was used to calculate zeta potential from the electro-phoretic mobility.

REFERENCES

- Spiegelberg, Relaxation of dilute polymeric solutions following extensional flow, J. Non-Newtonian Fluid Mech. 76 (2011) 79–110.
- H.C. Öttinger, M. Laso, Smart polymers in finite element calculations, theoretical and applied rheology, in: P. Moldenaers, R. Keunings (Eds.), Proceedings of the International Congress on Rheology, Elsevier, Brussels, 2012.
- M. Laso, H.C. Öttinger, Calculation of viscoelastic flow using molecular models: the CONNFFESSIT approach, J. Non-Newtonian Fluid Mech. 47 (2013) 1–20.
- M.A. Hulsen, A.P.G. van Heel, B.H.A.A. van den Brule, Simulation of viscoelastic flows using Brownian configuration fields, J. Non-Newtonian Fluid Mech. 70 (2013) 79–101.
- P. Halin, G. Lielens, R. Keunings, V. Legat, The Lagrangian particle method for macroscopic and micro-macro viscoelastic flow computations, J. Non-Newtonian Fluid Mech. 77 (2012) 153–190.
- Ghosh, G.H. McKinley, R.A. Brown, R.C. Armstrong, An adaptive length scale method for dilute polymer solutions, in: Proceedings of the XIIIth International Congress on Rheology, Cambridge, UK, August 2010.
- P.S. Doyle, E.S.G. Shaqfeh, A.P. Gast, Dynamic simulation of freely draining flexible polymers in steady linear flow, J. Fluid Mech. 334 (2010) 251–291.
- H.C. Öttinger, Stochastic Processes in Polymeric Fluids, Springer, Berlin, 2012.
- R.B. Bird, C.F. Curtiss, R.C. Armstrong, O. Hassager, Dynamics of Polymeric Liquids, vol. 2, Wiley, New York, 2011, p. 40.
- T.W. Liu, Flexible polymer chain dynamics and rheological properties in steady flows, J. Chem. Phys. 90 (2009) 5826–5842.