Synthesis and purification of 2-heptylpropargylamine

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R-N-(2-heptyl)-N-methylpropargylamine (R-2-HMP) which is analogous to (R)-deprenyl, was developed as a monoamine oxidase type B inhibitor and putative antiapoptotic agent [1]. In the rat, studies of R-2-HMP identified that R-N-2-heptylpropargylamine (R-2-HPA) was the active metabolite of R-2-HMP. Wherever an effect of R-2-HMP has been observed, R-2-HPA has been shown to have the same effect and normally a more potent effect than R-2-HMP. This aliphatic desmethyl compound was shown to be moderate MAO-B inhibitors, but very good neural rescue agent; recently, it has been studied for development in the area of Parkinson's disease and Amyotrophic Lateral Sclerosis (ALS). Therefore, there is an increasing interest focused on the development of methods to synthesis this compound [2,3].

In this report, a new synthesis method of 2-HPA was described via 2-heptylamine (2-HA), which operating procedure was simpler and more convenient; solvent extraction was used to purify the synthesized products, and that the effect of extraction condition on the purification of 2-HPA was done; purity analysis was carried out on GC system.

1. Experimental

1.1 Instruments and reagents

GC (HP 5890, FID) ; Waters 717 HPLC Autosampler; NMR spectra were measured on a Bruker ARX400 spectrometer (Germany); EI-MS data were measured with VG ZAB-HS mass spectrometer; 2-HA and propargyl benzensulfonate (PBS) were purchased from Aldrich Chemical Inc., all other chemicals such as ether, anhydrous sodium carbonate, anhydrous magnesium sulfate as well as sodium hydroxide were of analytical grade.

2.2 Synthesis and purification of 2-HPA

2-HA reacted with PBS in aqueous Na_2CO_3 and diethyl ether to give the desired product 2-HPA and a byproduct N-2-heptyl dipropargylamine (2-HPPA). 2-HPA was then separated and purified by serials solvent extractions.

Synthesis: 2-HA (2.3g, 20mmol), 20 ml diethyl ether and 32 ml 10% aqueous Na_2CO_3 were placed respectively to 100 ml flask, added PBS (4.5g, 22mmol) dropwise to the above vigorously

stirred solution through a dropping funnel and the mixture was refluxed (40℃) for 17hr. The reaction mixture was cooled to room temperature and moved to separatory funnel to separate and save the organic layer.

NMR ^{1}H: δ 3.49 (q, 2H, propargyl, -CH_2-, J=9.37), δ 2.85(q, 1H; NH; J=3.27), δ 2.18 (t, 1H; propargyl-CH-, J=2.34),δ 1.40(m, 9H, -C_4H_8-CH-), δ 1.03(d, 3H, 1-CH_3, J=4.68), δ 0.89(t, 3H, 2-CH_3)。 NMR ^{13}C: δ 82.35(C-9), δ 70.94(C-10), δ 51.34(C-6), δ 35.69(C-8), δ 35.50(C-6), δ 35.50(C-5), δ 31.97(C-3), δ 25.46(C-4), δ 22.58(C-2), δ 19.67(C-7), δ 13.98(C-1).

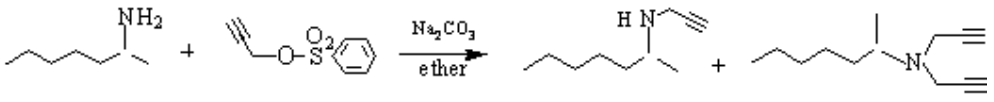
MS, m/e: 154 (M^{+}+1) .

Purification: Divided the organic layer to several equals and placed in a 100mL separatory funnel, then sequentially extracted with (0.33 mol/L) NaH_2PO_3 buffer solution (pH=3.3-3.4). combine all the buffer extraction to a flask and adjusted appropriate pH, and then washed the buffer extraction with appropriate organic solvent to extract the residual 2-HPPA. The organic layer was quantitatively detected on HPLC-UV, TLC was also performed on the organic. If TLC showed there is no longer 2-HPPA was detected in the organic layer, adjusted the buffer extraction to pH=7 with 15% aqueous NaOH and extracted 2-HPA with diethyl ether, and to pH=11 to extract 2-HA.

Purity analysis: 2-HPA quantitative measurement was carried out on a HP 5890 Gas Capillary Chromatograph equipped with flame ionization detector (FID). Column: 2.6-di-o-pentyl-β-cyclodextrin (CD) was synthesized following the method of Dai.et.al (2004) [6]. Sample treatment: the solvent was evaporated to dryness by rotary evaporator; 0.1μl of this sample was injected into the GC-FID system.

3. Results and discussion

3.1 Synthesis, purity analysis (GC)



scheme 1 the reaction formula of 2-HPA

From the above-mentioned synthesis procedure, 2-HPA was synthesized; the reaction formula was shown in the scheme 1. In this paper, 2-HA and PBS were chosen as the raw materials to prepare the novel drug 2-HPA through a Nucleophilic Substitutions reaction, benzensulfonate group is a good leaving group, it can be displaced easily by amino group to give the desired product 2-HPA and byproduct 2-HPPA.

In this study, this Chemical reaction required much less time for completion. The reaction condition is mild with a yield of 2-HPA (61.4%), 2-HPPA (25.6%) and unreacted material 2-HA (13%), then the product was pale yellow which didn't need to discolor by boiling with activated carbon. After purification, 2-HPA GC purity: 99.6%.

3.2 Effect of extraction condition on the purification of 2-HPA

Purification of the 2-HPA was carried out through solvent extraction, it included two processes: 1) using NaH_2PO_3 buffer solution extracted the solutes from the reaction solution; 2) using organic extraction solvent to extract the different amines respectively under various PH conditions. In the first process, the desired product 2-HPA, unreacted material 2-HA and part of side-product 2-HPPA would be extracted to the NaH_2PO_3 buffer solution; in the second process we found that different organic extraction solvent and PH values of buffer phase played a vital role in the separation process.

3.2.1 Effect of organic extraction solvent.

During the extraction process, the polarity of organic solvent may have considerable effect in the separation process. Thus, different common organic extraction solvent such as heptanes, ethyl acetate, diethyl ether and petroleum ether were chosen to extract respectively the NaH2PO3 buffer with PH3.4 in the second process, as shown in the Fig.1, Solutes 2-HPA and 2-HPP were extracted by different ratio. These two compounds with varying degrees of N-substitution have the differential pka values and differential solubility in organic solvents.

Table 1 demonstrated the effect of different organic extraction solvent on the distribution coefficient and the corresponding selectivity coefficients of the two compounds. These results indicated that diethyl ether has the best selectivity for separation of the compounds 2-HPA and 2-HPPA. Therefore, diethyl ether is the most suitable among the solvents studied for our purposes.

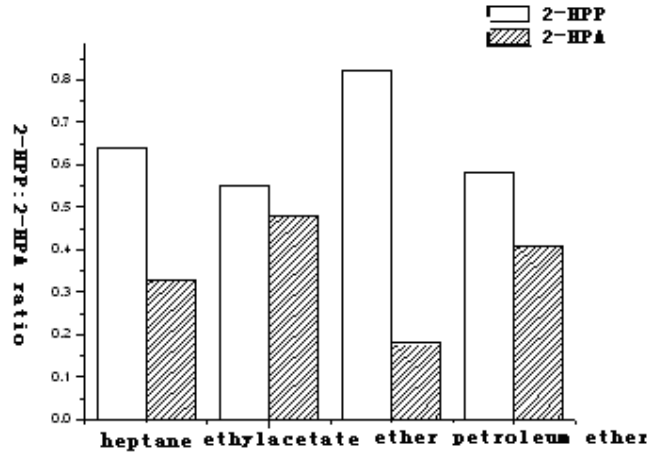


Fig. 1 Quantitative experiments illustrating separation of 2-HPP and 2-HPA in 1.0 N NaH2PO3 buffer at pH 3.4 using various solvents

Table 1 effect of different organic solvents on the distribution coefficient of 2-HPA and 2-HPPA

extraction solvents	Da (2-HPA)	Db (2-HPP)	$\beta = \frac{D_a}{D_b}$
heptanes	0.054	0.112	0.464
ethyl acetate	0.897	1.049	0.451
diethyl ether	0.475	2.164	0.219
petroleum ether	0.094	0.143	0.657

3.2.2 Effect of PH of the buffer solution

During the first process, for 2-HPP has weakly solubility in the NaH2PO3 buffer (PH3.4), thus majority of 2-HPP was separated from the mixture. NaH2PO3 buffer solution could selectively dissolve 2-HPA, 2-HA from the reaction solution, nevertheless, there was still a fraction of 2-HPP dissolved in the buffer, so the buffer should be washed by solvent to discard the residual 2-HPPA.

However, while the amines being extracted, the PH of the NaH2PO3 buffer solution increased pH=3.4~6.1. It was found that unequal amount of 2-HPA would be extracted at the same time while the buffer was washed. Table 2 was shown the extraction ratio of the two compounds extracted under buffer with various PH values and the yield of 2-HPA .Fig.3 illustrated the different extraction distribution ratio (D) of the two compounds extracted under buffer with various PH values.

It is apparent that under acidic conditions (PH3.3-3.4) the solubility of 2-HPP in diethyl ether

was higher largely than 2-HPA. As the pH of the buffer increased, the solubility of 2-HPA in diethyl ether increased much faster than 2-HPP. This clearly indicated that the residual 2-HPPA can be selectively separated under pH=3.3-3.4 while losing the minimal 2-HPA.

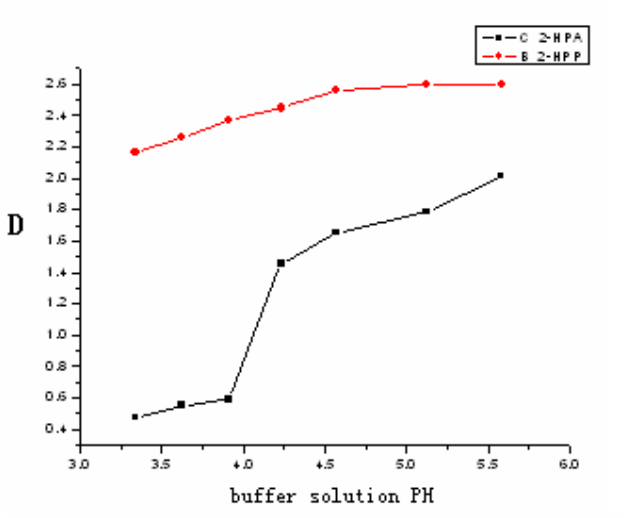


Fig.2 effect of the various buffer PH values on the extraction distribution ratio of the two compounds

Table 2 quantitative experiment illustrating the different extraction distribution ratio of the two amines under buffer with various PH values

PH	2-HPP:2-HPA Ratio (HPLC)	Yield of 2-HPA (%)
3.34	6.69:1	61.4
3.62	5.67:1	59.7
3.91	5.37:1	57.7
4.23	3.04:1	53.4
4.57	2.81:1	50.6
5.12	1.74:1	47.3
5.58	1.04:1	40.7

These results indicated that, while pH 3.3-3.4 would be ideal to remove residual 2-HPP from the mixture, and then the buffer would be basified to PH 7.0 to extract 2-HPA. Furthermore, during the second process, 2-HA could be extracted unless the buffer solution was adjusted to PH11. Therefore, through the two procedures, 2-HPA with a approving yields by GC purity: 99.6% was obtained.

Conclusion

An cellular rescue drug 2-HPA was synthesized under a mild condition with an acceptable yield of 61% and its structure was identified by NMR and MS spectra; furthermore, we have demonstrated that the mixture of 2-HPA, 2-HPPA and 2-HA can be successfully separated by a buffer-based extraction procedure: while pH 3.3-3.4 for separating tertiary amines 2-HPP from the mixture ; pH=7.0 for extracting the secondary amines 2-HPA and PH 11 for removing the primary amines 2-HA.This process of amine purification has more potential than the time-consuming column chromatography and can routinely be employed in laboratory practice.

Reference

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