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Communications

The Construction of Manual Vessel for SPPS and the Synthesis of a Dermorphine Analogue

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The reaction vessel of a peptide synthesizer is the heart of the instrument. Merrifield[1,2] introduced for the first time a shaker principle to mix the liquid and gel phase for this purpose. Several other instruments using mechanical stirring[3], gas bubbling[4], centrifugal system[5], simultaneous manual system[6] and rotary vessel[7] have been constructed in various laboratories and severals are available commercially. Our laboratory designed another new manual apparatus (Figure 1) with overhead-stirring arm for solid phase peptide synthesis (SPPS). From the view point of easy washing of polymer which is sticking to the wall of the vessel, easy displacement of vessel and no destruction of polymer resin, this overhead-stirring vessel is more convenient than other manual apparatus and also inexpensive. We confirmed that the overhead-stirring vessel was very convenient for manual solid phase peptide synthesis. It consists of 100 m*l* capacity glass cylinder (30 × 100 m*l*), a fritted filter disc embeded within the flask and one way stopcock, and also 1 mm (id) teflon tubing was used to connect the reagent reservoirs to the valve and reaction vessel. Glass rod was used to hold up the stirring bar, thereupon it was possible to agitate without grinding the resin. The mixing of the resin and the removal of the solvents was very efficient and the entire synthesis was carry out without opening the vessel. Moreover, the resin could be easily agitated with laboratory magnetic stirrer with the aid of several grooves on the wall of vessel and easily cooled under ice bath or other cooling system.

Synthetic program on dermorphine analogues, (Sar)^4-dermorphinoyl-Arg^8-Arg^9-Val^{10}-NH_2, has been under taken with the objectives of comparing their biological activity to the C-terminal free demorphine analgues[8]. And also, we are synthesizing eight other dermorphine analogues successfully for the same purpose now.

Synthetic Protocol. The synthesis was followed the stepwise solid phase strategy with the acid labile *t*-Boc group for the temporary N-protection of the amino acids and BMPI/HOBT reagent[9] as the coupling agent. Side chains of amino acids were protected by benzyl (Ser), Nitro (Arg) and 2,6-dichlorobenzyl (Tyr) groups respectively. The completness of each coupling steps were monitored by the Kaiser test[10]. For the purpose of introducing the first amino acid residue to the resin, Cesium salt method[11] was employed.

Cleavage of the Peptide from the Support and Identification. Following the stepwise coupling with BMPI/HOBT[9], the resin was suspended in anhydrous methanol/dioxane (1:9 v/v) below −10 *c* and stirred for three days, while a gentle stream of ammonia gas was passed through the reaction mixture. The oily product separated was dissolved in liquid ammonia and sodium was added until blue colour

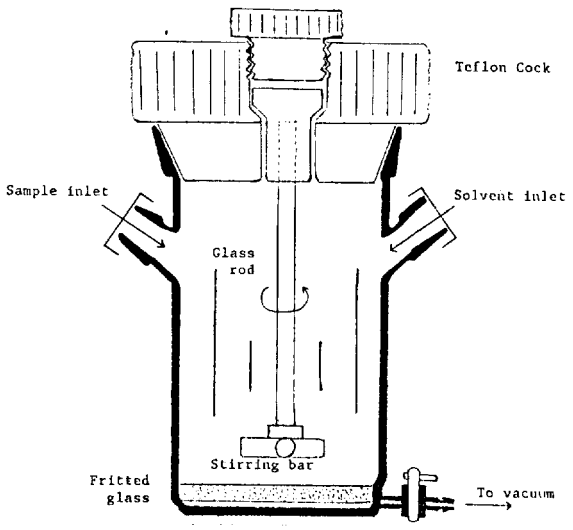

Figure 1. Diagramatic representation of the new manual over head-stirring vessel for SPPS.

remained for 15 minutes. A portion of the crude material, after evaporation, was dissolved in 0.1 N AcOH and subjected to gel filtration on a column (1.5 × 40 cm) of Sephadex G25 with 0.1 N AcOH. Cation exchange chromatography was done on a carboxymethyl cellulose column (Bio-Rad) eluted with 0.5 M ammonium acetate buffer (pH 7.0) solution. Analytical reverse phase HPLC of the product gave the following results: Retention time; 8.61 min, Purity; 91.1%, Fluent; 0.1 M NaH_2PO_4 (pH 6.9) in 25%MeCN/water, Amino Acid analysis; Ser 0.84 (1), Sar 0.96 (1), Pro 0.96 (1), Ala 1.04 (1), Val 0.90 (1), Tyr 1.82 (2), Phe 0.97 (1).

In conclusion, this type of reaction vessel for SPPS is proved to be another stationary reaction vessel which can be protected from atmospheric moisture. And, the vacuum applied in the reaction vessel can draw the next solvent or reagent into the vessel without inverting the reaction vessel[2,12], which allows for cooling the vessel with simple laboratory cooling system.

References

1. R. B. Merrifield and J. M. Stewart, *Nature*, **297**, 522 (1965).
2. R. B. Merrifield, L. Vizioli and H. G. Borman, *Biochemistry*, **21**, 5020 (1982).
3. Y. W. Kirby and P. K. Wirme, *Anal. Biochem.*, **85**, 307 (1978).
4. L. Corley, D. H. Sachs, and C. B. Anfinsen, *Biochem. and Biophy. Res. Comm.*, **47**, 1353 (1972).
5. C. Birr and W. Lochinger, *Synthesis*, 319 (1971).
6. J. J. Gorman, *Anal. Biochem.*, 136 (1984).
7. N. J. Hong, S. U. Koock and S. D. Shin, *Korea Biochem. J.*, **18**, 4109 (1985).
8. N. J. Hong, J. K. Jung and S. U. Koock, *Korean Biochem. J.*, **21**, 479 (1988).
9. N. J. Hong, S. K. Choi and S. U. Koock, *Bull. Korean Chem. Soc.*, **10**, 19 (1989).
10. E. Kaiser, R. L. Colesott and P. I. Cook, *Anal. Biochem.*, **34**, 595 (1970).
11. B. F. Gisin, *Helv. Acta.*, **56**, 1476 (1973).
12. J. M. Stewart and J. D. Young, "Solid Phase Peptide Synthesis", 2nd ed., p. 130, Pierce Chemical Company, Rockford, Illinois (1984).

Reaction of Thebaine with Trifluoromethyl Substituted Acetylenic Dienophiles

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In the search for more potent analgesics, the chemical modification of thebaine **1**, which is too toxic to be used as an analgesic, has been widely performed as one of better approaches.[1] Since thebaine **1** has the electron-rich diene moiety in the cyclic system, especially, a number of Diels-Alder reaction of thebaine **1** with a variety of dienophiles and chemical transformations of the resulting adducts have been extensively studied for a long time. In contrast to the numerous studies on the Diels-Alder reaction of thebaine **1** with olefinic dienophiles,[2-7] there have been a few reports relating the reaction of thebaine **1** with acetylenic dienophiles.[8-10] Rapoport and Sheldrick[8] reported that reaction of thebaine **1** with dimethyl acetylenedicarboxylate (DMAD) in benzene at 50 °C provided the Diels-Alder adduct in 90% yield, while the similar reaction of ethyl propiolate (EP) afforded the Diels-Alder adduct in 6% yield. Recently, Kanematsu[9] and Archer[10] independently found that the addition reactions of thebaine **1** with acetylenic dienophiles remarkably depended on the solvent and provided new 1:1 addition products in polar solvents.

As one of our efforts to obtain morphine alkaloid of biological interest from thebaine **1**, we are interested in the introduction of trifluoromethyl group into the thebaine system *via* reactions of thebaine **1** with trifluoromethyl substituted acetylenic dienophiles because of unique nature of trifluoromethyl group for biological activities. It has been well known that the presence of trifluoromethyl moiety often confers unique properties to a molecule in terms of increased lipophilicity.[11] In this communication we address the reactions of thebaine **1** with trifluoromethyl substituted acetylenic dienophiles **2a-b**, which are easily prepared from the previous methods,[12,13] in nonpolar and polar solvents and also examine the effect of trifluoromethyl group in the reaction.

When thebaine **1** was allowed to react with trifluoropropyne **2a** in benzene at room temperature for 18 hours, only 1:1 addition adduct **3** was obtained in 34% yield: mp 79-80 °C; mass spectrum, *m/e* 405 (M^+); IR (KBr) 1670, 1720 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.93 (m, 2H, H-15), 2.94 (s, 3H, NCH_3), 3.19-3.28 (m, 4H, H-10, H-16), 3.57 (s, 3H, 6-OCH_3), 3.84 (s, 3H, 3-OCH_3), 4.52 (d, 1H, H-8, J = 5.0 Hz), 5.00 (s, 1H, H-5), 5.15 (d, 1H, H-7, J = 4.9 Hz), 5.94 (d, 1H, H-9, J = 5.2 Hz), 6.66 (d, 1H, H-1, J = 8.0 Hz), 6.69 (d, 1H, H-2, J = 8.0 Hz) 7.35 (s, 1H, H-18); ^{19}F NMR ($CHCl_3$) -65.52(s). No Diels-Alder adduct was obtained. However, remarkable increase of yield (~95%) for the addition adduct **3** was accomplished by using acetonitrile as a solvent in the reaction. When the same reaction was carried out in methanol as a solvent, methanol-addition adduct **4** was obtained in 60% yield: mp 56-58 °C; mass spectrum, *m/e* 437 (M^+); IR (KBr) 1675, 1725 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.81 (m, 2H, H-15), 2.93 (s, 3H, NCH_3), 3.07-3.22 (m, 2H, H-16), 3.28 (dd, 1H, H-10α, J = 6.3, 20.0 Hz), 3.44 (d, 1H, H-10β, J = 19.4 Hz), 3.54 (s, 3H, 6-OCH_3), 3.64 (s, 3H, 6-OCH_3), 3.90 (s, 3H, 3-OCH_3), 4.43 (d, 1H, H-19, J = 12.9 Hz), 4.78 (s, 1H, H-5), 5.61 (d, 1H, H-7, J = 9.9 Hz), 5.95 (d, 1H, H-9, J = 5.0 Hz), 6.61 (d, 1H, H-8, J = 9.9 Hz), 6.66 (d, 1H, H-1, J = 8.1 Hz), 6.70 (d, 1H, H-2, J = 8.1 Hz), 7.34 (d, 1H, H-18, J = 12.9 Hz). ^{19}F NMR spectrum was not taken because the adduct **4** was easily decomposed at room temperature in 24 hours. The coupling constant of the H-18 and H-19 protons (J = 12.9 Hz) indicates that the stereochemistry of double