Arch. Pharm. Res. **15**(2), 184-186 (1992)

Malonyl Amino Acids and Their Esters as Psychoactive Agents I

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(Received October 2, 1991)

Abstract □ Malonic acid amides were synthesized using different amino acids and their esters. The synthesized compounds were evaluated for their sedative activity on rats. Potentiating effect of all the compounds on pentobarbitone induced sleep on rats was observed. Plasma protein binding studies were also carried out and it was observed that the synthesized compounds have low plasma protein binding as compared to barbiturates.

Keywords □ Malonic acid, arginine, glycine, alanine

Barbituric acid (2,4,6-trioxohexahydropyrimidine) lacks central depressant activity, but the presence of alkyl or aryl groups at position 5 confers sedative hypnotic activity and sometimes other activities. In general, structural changes that increase lipid solubility decreases duration of action, latency to onset of activity, accelerate metabolic degradation, and often increase hypnotic potency. Thus large aliphatic groups at C-5 confer greater activity than do methyl groups, but the compounds have a shorter duration of action. Introduction of polar groups, such as ether, keto, hydroxyl, amino or carboxyl groups, into alkyl side chains decreases lipid solubility and abolished hypnotic activity. Methylation of 1-N atom increases lipid solubility and shortens duration of action, although demethylation to a longer acting metabolite may occur. Lipid solubility also favours interaction with hydrophobic regions in proteins. It correlates roughly with binding to plasma protein and cytochrome.[1] Sedative-hypnotic use of barbiturates suffers various side effects, viz. dependence liability, enzyme induction and tolerance. Various methods have been used to reduce these side effects.[2]

In this study an attempt has been made to design and evaluate compounds having pharmacopheric groups which are essential for sedative hypnotic activity.

EXPERIMENTAL METHODS

General procedure for the Synthesis

The compounds were synthesized in the following steps: Utilizing the process of Ronald[3], methyl ester hydrochlorides of glycine and L-alanine were synthesized. Amino acid (0.05 mol) was added to a solution of thionyl chloride (0.05 mol) in methanol and the reaction mixture was then stirred under reflux for 4 hours. The solvent was removed under reduced pressure and residue was triturated with several 20 m*l* portions of cold ether at 0℃. The crude product was recrystallized from hot methanol (25 m*l*) by slow addition of ether (150-220 m*l*) followed by cooling at 0℃. L-alanine methyl ester hydrochloride (79% yield, mp. 153-155°) DL-glycine methyl ester hydrochloride (yield 87%, mp. 159-160°) were thus synthesized.

Malonyl dichloride

Malonyl dichloride was prepared by method reported earlier.[4] Malonic acid amides from amino acid ester hydrochloride and malonyl dichloride: The procedure based on modified Schotten Baumen reaction[5] was adopted.

N-N Malonyl bi (methyl glycinate)

Glycine methyl ester hydrochloride (0.09 mol) was

Table I. Characteristics of substituted malonic acid derivatives
General structure CH_2 $CONHR'\approx$ $CONHR'\approx$

S. No.	Compound	R^1	Molecular Formula	Appearence	Yield %	M.P./B.P. ℃
1.	**1**	$-CH_2COOCH_3$	$C_9H_{14}N_2O_6$	White Crystals	72	167-169
2.	**2**	$-\underset{\displaystyle CH_3}{\overset{\displaystyle |}{C}}HCOOCH_3$	$C_{11}H_{18}N_2O_6$	Colorless Liquid	68	61-68 (B.P.)
3.	**3**	$-CH_2COOH$	$C_7H_{10}N_2O_6$	White Crystals	82	156-158
4.	**4**	$-\underset{\displaystyle CH_3}{\overset{\displaystyle |}{C}}HCOOH$	$C_9H_{14}N_2O_6$	White Crystals	76	150-151
5.	**5**	$-\underset{\displaystyle NH}{\overset{\displaystyle \|}{C}}NH(CH_2)_3\underset{\displaystyle NH_2}{\overset{\displaystyle COOH}{\overset{\diagup}{\underset{\diagdown}{CH}}}}$	$C_{15}H_{28}N_8O_6$	Colorless Liquid	63	135-137(B.P.)

*All compounds were analysed for C, H and N. All values were within±0.5% of the theoretical value. The IR spectra in KBr phase showed the presence of amide linkage in all compounds. The M.P. was determined in open capillaries and are uncorrected.

Table II. Hypnotic activity

S. No.	Compound	Dose mg/kg b.w.	Mean sleeping time	p-Value
1.	**1**	40	53.75±0.0372*	0.05
2.	**2**	40	53.00±10.0166$^+$	0.1
3.	**3**	40	36.25±3.7881$^+$	0.2
4.	**4**	40	38.00±4.4581$^+$	0.1
5.	**5**	40	49.75±8.4571*	0.05
6.	Control	30	25.75±4.2920	

*The difference was found to be statistically significant ($p<0.05$) when compared with control.
$^+$The difference was found to be statistically in-significant when compared to control ($p>0.05$)

dissolved in 25 m*l* distilled water, potassium carbonate (10%) was added to the above solution dropwise with stirring at temperature below 10℃ until effervescence ceased. To this solution malonyl dichloride (0.05 mol) was added slowly and the reaction mixture was kept for 4 hours at room temperature. The reaction mixture was evaporated at temprature below 70℃. After adjusting pH at 7.0 the compound was precipitated by adding acetone to the concentrated reaction mixture.

N-N Malonyl bi (methyl alanate) was prepared similarly using 0.05 mol malonyl dichloride. N-N malonyl bi (glycine), N-N Malonyl bi (alanine) and N-N Malonyl bi (arginine) were prepared similarly using malonyl dichloride (0.05 mol) and respective amino acid (0.1 mol). The purity of synthesized compounds was checked by TLC. The physical data of these compounds are listed in Table I.

EVALUATION

Effect on central Nervous System (Effect on pentobarbitone induced sleeping time of rats)

Albino rats weighing 100-200 g were used in this study. The animals were divided into 6 groups, each group having 6 animals. The first five groups were administered with the synthesized compound at the dose of 40 mg/kg body weight intraperitoneally. After 20 minutes of the drug administration, pentobarbitione sodium at the dose of 30 mg/kg body weight was given intraperitoneally to the above five groups, sixth group served as control. The results were anal-

Table III. Percent plasma protein binding

Compound	1	2	3	4	5
% Plasma protein binding	22	30	26	32	20

ysed statastically by using student 't' test.[6] The results are shown in Table II.

Plasma protein binding studies

A solution of synthesized compounds (50 μg/ml) was prepared in phosphate buffer pH 7.4, 100 ml of this solution was used for the study. The prepared cellophane membrane was tied at one mouth of the dialysis tube, which was dipped into drug solution and was covered. The whole assembly was placed on a magnetic stirrer at low rpm. The temperature was maintained at 37±0.5°.[7] After every 2 hours samples were withdrawn and assayed spectrophotometrically. The results are shown in Table III.

RESULTS

The results of hypnotic activity studies showed that the synthesized compounds have hypnotic activity. Although all the compounds potentiated the pentobarbitone induced sleeping time, compound **1** and **5** showed better activity. Plasma protein binding studies showed that the synthesized compounds have very low plasma protein binding as compared to barbiturates.

CONCLUSION

In the present study an attempt was made to overcome the serious side effects of barbiturates by synthesizing malonyl amino acids and their esters. These compounds are acyclic and showed good sedative activity. However, further studies should be carried out to obtain promising compounds without side effects.

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