ANALGESICS AND THEIR ANTAGONISTS: SOME STERIC AND CHEMICAL CONSIDERATIONS

PART II. THE INFLUENCE OF THE BASIC GROUP ON PHYSICO-CHEMICAL PROPERTIES AND THE ACTIVITY OF METHADONE AND THIAMBUTENE-TYPE COMPOUNDS

BY A. H. BECKETT, A. F. CASY, N. J. HARPER AND P. M. PHILLIPS

From the Chelsea School of Pharmacy, Chelsea Polytechnic, London, S.W.3

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NO relation could be shown to exist between the analgesic activities of centrally acting analgesics and their dissociation constants[1]. However, minor modifications of the basic group in analgesics vary their activity[2–4] and it has been thought that association occurs between the basic group of the drugs and the anionic site of the analgesic receptor[5]. We are now concerned to measure the effect of changes in the basic group upon this association.

The factors contributing to the strength of the attractive forces between the basic group (as a cation) and the anionic site may be listed. They are, the strength of the base, the spatial arrangement of the basic centre in relation to the flat aromatic ring in the molecule, the size of the basic group, and the presence, in the groups attached to the cation, of groups or atoms with dipoles which introduce further attractive forces between the drug and the anionic site.

MEASUREMENTS

A measure of the bulk of the various cationic groups present in analgesics was made. Molecular models (Catalin) were constructed employing a quaternary nitrogen atom. The approximate width of the basic group was then measured at right angles to the plane joining the cationic head to the rest of the molecule. Piperidino and morpholino compounds were constructed in the chair conformation with equatorially orientated ring methyl groups in cases of the mono methyl substituted rings. The 2:6-dimethyl piperidino group was constructed with both groups equatorially orientated (the meso compound).

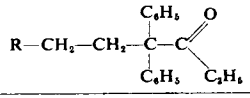
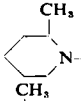
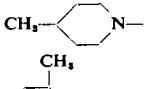
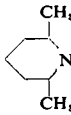
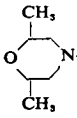
Some doubt exists about the correct measure of the effective "width" of the dialkylamino or aralkylamino groups because of the puckering and rotation of these alkyl chains. For the present purpose, the effective width is presumed to be the minimum width consistent with free rotation of the alkyl chains.

Methadone-type compounds

The effective widths, expressed in Ångström units. of normethadone compounds (II) are recorded in Table I along with the analgesic activities of the compounds in rats (activities compared against pethidine). It is well known that the relative activities of different analgesics vary in different animals and low activities are difficult to measure with accuracy.

TABLE I

ANALGESIC ACTIVITIES AND EFFECTIVE WIDTHS OF THE BASIC GROUP OF METHADONE-TYPE COMPOUNDS. (COMPOUNDS NOS. 5 AND 6 WERE PREPARED AND TESTED BY MORRISON AND RINDERKNECHT[12], THE REMAINDER BY DUPRÉ AND OTHERS[11].)

Compound No.	 R	Analgesic activity (in rats) (pethidine = 1)	Approx. width of basic group in Ångström units
1	$(CH_3)_2N$—	1	5·4
2	$(C_2H_5)_2N$—	½–1	6·7
3	$(n\text{-}C_3H_7)_2N$—	0–⅓	7·3
4	$(n\text{-}C_4H_9)_2N$—	0–⅓	8·3
5	$CH_2=CHCH_2NCH_3$	½	6·3
6	$(CH_3=CHCH_2)_2N$—	1	7·8
7	$(PhCH_2)_2N$—	—	
8	$(PhCH_2)N\cdot CH_3$	0	8·4
9		4	5·6
10		2–3	6·0
11		0	7·3
12		0–⅓	7·3
13		3	6·0
14		0–⅓	8·4
15		7	6·1
16		1½	7·5
17		1½	8·4

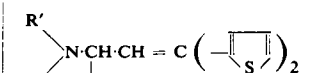
However, the activities quoted in Table I, and especially those used in Figure 1, most of which were obtained in one laboratory[11] may be used as a basis for broad generalisations about structure-activity relationships.

Thiambutene-type compounds

The analgesic activities of a series of these compounds and the effective width of their basic groups are recorded in Table II. The dissociation constants of certain of these compounds is given in Table III. The prep-

TABLE II

ANALGESIC ACTIVITIES AND EFFECTIVE WIDTHS OF THE BASIC GROUP OF THIAMBUTENE-TYPE COMPOUNDS

Compound No.	 R'R"N	Analgesic activities* morphine sulphate = 1	Approx. width of basic group in Ångström units
18	$(CH_3)_2N$—	1·07 (*b*)	5·4
19	$CH_3N{\cdot}C_2H_5$	1·7 (*b*)	5·8
20	$CH_3N{\cdot}n{\cdot}C_3H_7$	0·1 (*b*)	6·4
21	$CH_3N{\cdot}iso{\cdot}C_3H_7$	0·3 (*b*)	6·5
22	$CH_3N{\cdot}CH_2C_6H_5$	0 (*b*)	6·3
23	$(C_2H_5)_2N$—	1·0 (*b*)	6·7
24	$(n{\cdot}C_3H_7)_2N$—	0 (*b*)	7·3
25	$(CH_2{=}CHCH_2)_2N$—	0·3 (*b*)	7·8
26		0·7 (*b*)	5·6
27		1·1 (*b*)	6·0
28		0·5 to 0·6 (*a*)	7·3
29		0·6 to 0·9 (*a*)	7·3
30		0·6 to 0·9 (*a*)	6·0
31	$(CH_2)_4N$—	1·3 to 1·8 (*a*)	6·6
32	$(CH_2)_7N$—	0·05 to 0·10 (*a*)	6·9

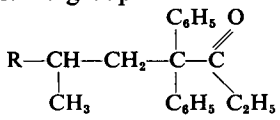
* Analgesic activities were determined in rats using heat and pressure methods; (*a*) present work, (*b*) abstracted from publication of Green[13].

aration of the compounds and the measurement of dissociation constants is described later in this paper.

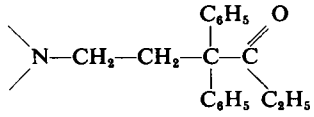
DISCUSSION

Methadone-type compounds

The replacement of the $-N(CH_3)_2$ group of methadone (I; R = $-N(CH_3)_2$) by $-N(C_2H_5)_2$ is attended by a considerable loss of activity[6]. If the 2 ethyl groups are joined to form a pyrrolidine ring, however, the analgesic activity is restored to the level of that of methadone[7]. The potency is retained upon replacing the basic group of methadone by a piperidino group[8]



(I)



(II)

and enhanced by a morpholino group[9]. Further increase in the ring size (I; R = $-N(CH_2)_6$ results in loss of activity[10]. Compounds of type II also exhibit similar variations in activity upon comparable changes in the basic group[11,12] (see Table I); a graphical representation of activities against width of the basic group is shown in Figure 1.

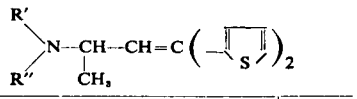
A simple relation between effective width of the basic group and analgesic activities is not apparent until the basic groups are divided into three different types, (*a*) those possessing alkyl or aralkyl substituents on the nitrogen atom, (*b*) those consisting of a heterocyclic ring other than morpholino, and (*c*) morpholino derivatives.

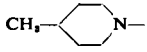
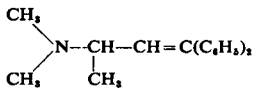
For types (*a*) and (*b*), two straight lines possessing different slopes represent the relationship between activities and width of basic group; activity is inversely proportional to width. Insufficient points are present to completely establish the relation involving morpholino compounds; it is reasonable to assume that once again a straight line relation exists as shown in Figure 1. The three lines intersect the "width" axis at approximately the same position corresponding to a width of basic group of 7·4 to 8·4 Å; analgesic activity in methadone type compounds possessing basic groups of larger effective width therefore seems unlikely.

TABLE III

DISSOCIATION CONSTANTS OF THIAMBUTENE-TYPE COMPOUNDS

SOLVENT, WATER; TEMP. 25° C.



No.		pK'a
18	$(CH_3)_2N$—	8·95
23	$(C_2H_5)_2N$—	8·90
27		8·46
28		7·46
29		8·28
30		8·20
31	$(CH_2)_6N$—	7·96
32	$(CH_2)_7N$—	7·52
33		9·26

The division of the basic groups into the three classes as shown appears logical on structural grounds if association of the cation with an anionic receptor site is an integral part of the total drug-receptor binding forces. If steric limitations near the anionic site are absent, van der Waals' forces between the receptor and the groups attached to the cationic head will reinforce the ionic bond. A complementary configuration of cationic head and anionic site will assist in the formation of this reinforced ionic bond. A piperidino and morpholino group may be regarded as equivalent in the steric sense; in the latter, the oxygen atom with its electron donating potentialities could well give rise to additional binding forces upon close contact with the receptor. The difference in dissociation constants of compounds containing the two types of group[1] is a further reason for the above separate classification of these basic groups in a consideration of analgesic activities. The classification of pyrrolidines (same dissociation constants as piperidines) and larger rings along with the piperidines is justified since although minor differences in the conformations of these rings exist, they would present roughly comparable surfaces to a receptor.

Increase in the effective width of the basic group is caused by the introduction of methyl groups into the 2 and 3 positions but not the 4 position of the 6-membered rings. The results (see Table I and Fig. 1) indicate that the incorporation of the cationic head into a ring structure is advantageous, presumably due to the increased van der Waals' force bonding between cationic head and receptor. However, steric requirements of the groups in the vicinity of the anionic site lead to reduction in activity with increasing width of the cationic head.

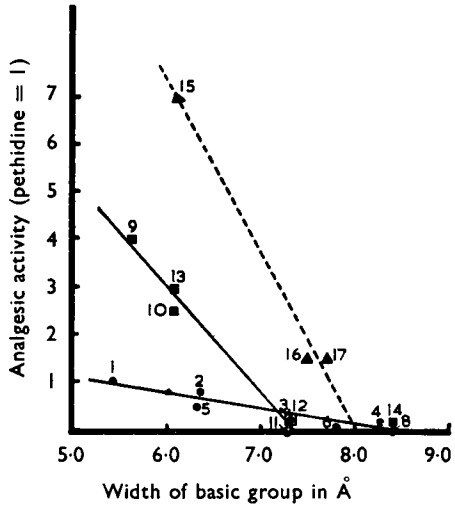


FIG. 1. Relationship between analgesic activity and width of basic group in methadone-type compounds. Compound numbers correspond to those in Table I.

● Alkyl or aralkyl group.
■ Heterocyclic group other than morpholino.
▲ Morpholino type group.

The dialkylamino groups are distinct from the above types, since separate rotation of the two alkyl groups is possible; the cationic head becomes less compact and less strong van der Waals' force bonding between the groups and the site would be expected. The reduced activity of these compounds, other than dimethyl, is attributed to this factor since the dissociation constants of dialkylamines, pyrrolidines and piperidines do not differ greatly. Increase in alkyl chain length results in a large increase in the effective width of the cationic head.

From these results it is possible to make the following observations: the ionic bond between cation and anionic receptor can be reinforced by van der Waals' bonding if the cationic head is suitably orientated in relation to the rest of the analgesic molecule; steric requirements in the vicinity of the anionic site reduce the bonding forces upon increase in effective width of the cation and completely prevent drug-receptor bonding if a width of approximately 8·0 Å is exceeded; increase in the dimensions of the groups attached to the *N*-atom along the axis between this atom and the rest of the molecule does not decrease the bonding forces (there is evidence to suggest that the bonding forces are increased—see Part III[14]).

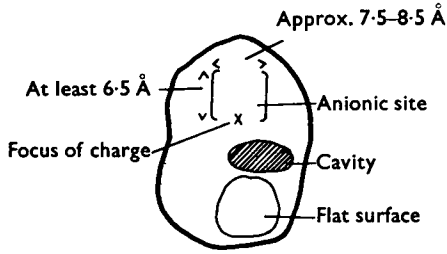
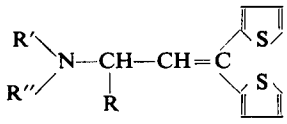


FIG. 2. Analgesic receptor site.

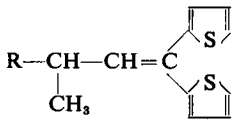
It may be concluded that the anionic receptor site has certain dimensions, and if the cationic head of an analgesic type molecule exceeds these, weaker association between the drug and the receptor results, with concomitant reduction in analgesic response. The analgesic receptor surface may now therefore be represented as in Figure 2.

Thiambutene-type compounds

Dithienylbutenylamines of type III exhibit powerful analgesic activity[14–16], and some are known to be antagonised by *N*-allylnormorphine[17,18]. It is reasonable to assume that they are adsorbed upon the same receptor surface as morphine, methadone and other analgesics, that they exist as semi-rigid structures, and that they adopt conformations similar to those of methadone-type compounds in solution (see Part I[1]).



(III)

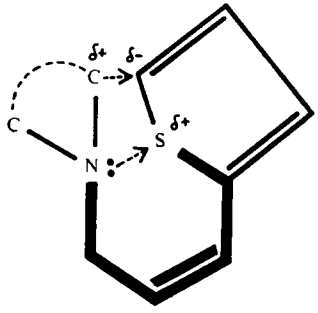


(IV)

If these conclusions are correct, the "effective width" of the basic group should influence the activity in a way comparable with that shown by the methadone-type compounds. Dissociation constants should provide information concerning conformations in solution although it is recognised that both inter- as well as intra-molecular interactions can affect the values. To test these conclusions, compounds of type IV, in which R consists of various amino groups, have been investigated.

Conformation of the molecules

The sulphur atom of a thiophen ring carries a partial positive charge due to resonance effects[19]. In agreement with Gero[20], it is considered that the two sulphur atoms in thiambutene-type compounds will tend towards positions of maximum separation due to steric and electrical repulsions. The double bond will contribute to the rigidity of the structure and the basic group will align itself in close proximity to one of the thiophen rings shown in V.



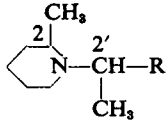
(V)

Gero[20] has emphasised that such a structure "imitates a piperidine ring", the ring being formed of N, S and the heavily outlined portion of (V). We believe the simulation of the piperidine ring to be unimportant in itself, but the above interactions stabilise the molecule in such a conformation that the basic centre, and the thiophen ring not involved in these interactions, are correctly orientated to allow ready association with the anionic site and flat portion respectively of the proposed "analgesic receptor site" (see Beckett and Casy[5] and Part I[1] of this series).

The adoption of such a conformation by this type of molecule will result in compounds, produced by varying the basic group, being more weakly basic than the corresponding simple *N*-alkylated compounds, due to the reduction of the electron density by the electrical effect and the steric limitations in the vicinity of the N atom[1]. The compounds will be weaker bases than predictable from considerations of inductive effect along the chain of atoms between the N atom and the thienyl groups.

Elsewhere[1] it was shown that compounds containing dimethylamino groups were stronger bases than the corresponding piperidino compounds by about 0·35 pKa units. Consideration of molecular models of dithienylbutenylamines in the conformation shown in V indicated that basic groups constituting part of a ring will probably be sufficiently close to one of the thienyl rings to permit strong van der Waals' force bonding. This will increase the steric limitations near the N atom (base weakening effect) to a greater extent than in the corresponding methadone-type compounds; such steric effects will be further increased upon introducing methyl groups into the heterocyclic ring, especially in the α-position. Dialkylamino groups will be expected to have a reduced steric requirement since the group *as a whole* will be less firmly bound by the thienyl group and cation formation should consequently be less hindered by steric factors.

The dissociation constants of the series of dithienyl-butenylamines recorded in Table III substantiate the above predictions. Compound 33 (thienyl groups of thiambutene-type compound replaced by phenyl groups) is a stronger base (0·31 pKa units) than compound 18—the electrical effects shown in V being absent in compound 33. (The alternative explanation that the difference is due to the greater −I effect of two thienyl groups than two phenyl groups operating through a carbon chain of three atoms is inadequate.) Compound 18 is as strong a base as methadone (pK′a 8·99) but the piperidino compound 27, and especially the substituted piperidino compounds 28, 29 and 30, are weaker bases than the piperidino analogue of methadone (pK′a 8·58). Since it has been shown that the introduction of a CH_3-group into the ring of an *N*-alkyl piperidine has a base strengthening effect (+I effect of CH_3-group is a more important factor than its steric requirements near the N atom in these compounds—see Part I) the △pKa values between compounds 27 and 28 can be attributed to increased steric limitations about the N atom in this latter compound. Only if the N atom is near the S atom in III; (R = CH_3), and the basic group as a whole is in close proximity to one of the thienyl rings, will such steric limitations occur in these compounds. The alternative explanation that, in compounds of type VI, the interaction of the base strengthening 2–CH_3 group (△pKa 0·3) and the base weakening 2′–CH_3 group (△pKa 0·3) (see Part I[1]) might lead to a large base weakening steric effect is excluded since it



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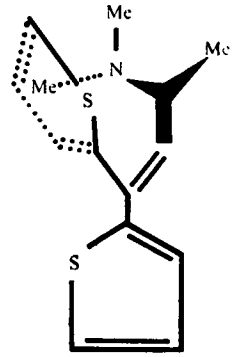
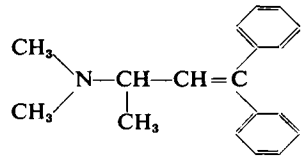


(VII)

does not occur in simple compounds of this type[21]. The weakly basic character of compounds 31 and 32 is explicable in the same terms. It is therefore concluded that thiambutene-type molecules in solution adopt the conformation depicted in VII, so that a surface complementary to that of the analgesic receptor site (see Fig. 2) is present in this molecular conformation.

This conclusion is supported by the fact that compound VIII, in which the electrical forces responsible for holding the basic group in the correct orientation (as in VII) are absent, has negligible analgesic activity[22].



(VIII)

Effective width of the basic group and analgesic activities

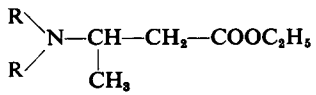
The analgesic activities of a series of thiambutene compounds and the effective width of their basic groups are recorded in Table II. The dissociation constants of certain of these analgesics indicate that no simple relation is possible between basic strength and analgesic activity. As in the case of the methadone-type compounds, the results indicate that there is some relation between the effective width of the cationic head and activity which is reduced upon increasing the width of the basic group. The pattern is not so consistent as observed for the methadone-type compounds (see Fig. 3).

Probably the greater $\triangle$pKa values between members of the thiambutene-type series (see Table III), in contrast to the methadone type, may be responsible for the reduced clarity of correlation, since the stability of the drug-receptor complex may be influenced by the strength of the base as well as the size.

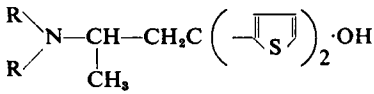
These results seem to provide further evidence for the adsorption of analgesics upon a common analgesic receptor incorporating an anionic site of circumscribed dimensions as indicated in Figure 2.

Preparation of the compounds

Compounds 18, 23 and 27 (Table III) have been described elsewhere[15]. The further compounds required were prepared by the condensation of the appropriate secondary bases with ethyl crotonate to yield the amino esters (IX) which, upon treatment with thienyl-lithium according to the method of Adamson[15], yielded the 3-amino-1 : 1-di(2′-thienyl)butan-1-ols (X). Dehydration of these amino-alcohols with dry hydrogen chloride



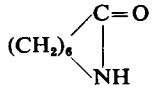
(IX)



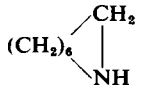
(X)

gave the corresponding 3-amino-1 : 1-di(2′-thienyl)-but-1-enes. (Table III, Compounds 28, 29, 30, 31, 32.)

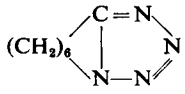
The secondary bases used above, with the exception of heptamethyleneimine (XII), were commercially available. This base was prepared by the reduction of *cyclo*heptanone isoxime (XI) with lithium aluminium hydride



(XI)



(XII)



(XIII)

according to the method of Ruzicka and others[23]. These workers described two methods for the preparation of the isoxime; the rearrangement of *cyclo*heptanone oxime by means of concentrated sulphuric acid and the treatment of *cyclo*heptanone with hydrazoic acid. The latter method was recommended since the former was too vigorous and gave only low yields of the isoxime. In the hands of the present authors, the treatment of *cyclo*heptanone with hydrazoic acid gave largely 4:5-*cyclo*hexamethylene tetrazole (XIII) together with only a small yield (about 20 per cent.) of the desired isoxime. Rearrangement of *cyclo*heptanone oxime, however, according to the method of Wallach[24] gave the isoxime (XI) in 70 per cent. yield.

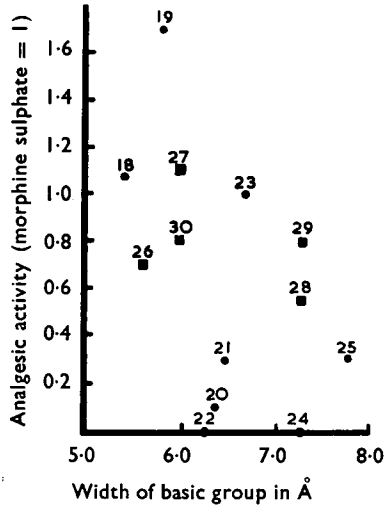


FIG. 3. Relationship between analgesic activity and width of basic group in thiambutene-type compounds. Compound numbers correspond to those in Tables II and III.

● Alkyl or aralkyl group.
■ Heterocyclic group other than morpholino.

Measurement of dissociation constants

In certain analgesic type compounds, e.g., methadone, the dissociation constant of the basic group is affected by solvent transmitted intramolecular electrical interactions between groups (see Part I)[1]. In other analgesics, e.g., morphine, the importance of such effects is reduced by the steric character of the molecule. Since the magnitude of these solvent transmitted effects is influenced by the dielectric constant of the medium, alteration of the composition of the solvent will not cause parallel effects upon the dissociation constants of both types of compound, e.g., Hall[25] found that the pK′a value in water of certain bases was a good index of base strength in organic solvents within a particular class, but compounds containing polar groups near the N atom belonged to a different class than those without such groups.

The low water solubility of many of these bases rendered impossible the titration of their salts to the point of half neutralisation. The use of

ethanol etc. to increase the solubility was inadmissible because of the above-mentioned factors; titrations at several concentrations and the extrapolation of the results to zero concentration of organic solvent was too time-consuming and wasteful in materials. However, it was possible to derive a relationship which permitted the calculation of pK'a values after titration to the point at which precipitation of the free base began.

EXPERIMENTAL

All m.pts. are uncorrected.

Microanalyses were by Mr. G. S. Crouch, School of Pharmacy, University of London.

Equivalent weights of the bases and picrates were determined by titration with 0·02 N perchloric acid in glacial acetic acid using Oracet Blue B[26] as indicator. Titration of the hydrohalide salts was carried out in non-aqueous media in the presence of mercuric acetate by the method described by Pifer and Wollish[27].

Cyclo*Heptanone isoxime*

(*a*) *From* cyclo*heptanone and hydrazoic acid.* A mixture of *cyclo*heptanone (25 g.) and hydrazoic acid (10·6 g.) in benzene (200 ml.) was added dropwise to a stirred, ice-cooled mixture of concentrated sulphuric acid (69 ml.) and benzene (100 ml.). The next morning, the mixture was made alkaline with strong aqueous sodium hydroxide and the precipitated oil extracted with chloroform. After drying (Na_2SO_4), the solvent was removed and the oil fractionally distilled to give the isoxime (5·3 g.) b.pt. 168° to 188° C./20 mm. and a residue of crude 4:5-*cyclo*hexamethylenetetrazole (7 g.), m.pt. 67° to 68° C. after recrystallisation from benzene (Ruzicka and others[28] give m.pt. 66° to 68° C.).

(*b*) *From* cyclo*heptanone oxime.* A mixture of *cyclo*heptanone oxime (5 g.), prepared by the method of Ruzicka and others[23], glacial acetic acid (5 ml.) and concentrated sulphuric acid (10 ml.) was gently heated until a lively reaction took place and the mixture boiled and turned black. Four more 5 g. batches of the oxime were treated in this way, the products bulked, made alkaline with ice-cold, strong aqueous sodium hydroxide, and the precipitated oil extracted with ether-chloroform. After drying (Na_2SO_4) the solvent was removed and the residue distilled to give the isoxime (18 g.) b.pt. 122° C./3 mm.

Heptamethylenimine

*cyclo*Heptanone isoxime (20 g.) in ether (20 ml.) was added dropwise, in the course of 50 minutes, to a stirred suspension of lithium aluminium hydride (8 g.) in ether (200 ml.), and the mixture refluxed for 3 hours. Excess of reagent was then decomposed with water and the mixture filtered. After drying (Na_2SO_4) the ether was removed and the residue distilled to give heptamethylenimine (12·6 g.) b.pt. 52° to 53° C./15 mm., n_D^{17} 1·4752, which gave a picrate, yellow needles, from ethanol-water, m.pt. 149° to 150° C. (Ruzicka and others[23] give n_D^{21} 1·4740 and m.pt. 147° C. for the picrate).

Ethyl 3-aminobutyrates (IX)

(*a*) (*IX* (*a*)). A mixture of hexamethylenimine (9·9 g.) and ethyl crotonate (11·4 g.) was refluxed for 3 hours and then fractionally distilled giving the amino-ester (IX (*a*)) (7·8 g.) b.pt. 142° to 144° C./15 mm., equiv. wt. 217 (calc. 213). It gave a *picrate*, yellow needles from ethanol, m.pt. 99° to 100° C. Found: C, 48·9; H, 5·9; N, 12·7. $C_{18}H_{26}O_9N_4$ requires C, 48·9; H, 5·9; N, 12·7 per cent.

(*b*) (*IX* (*b*)). A mixture of heptamethylenimine (11·3 g.) and ethyl crotonate (11·4 g.) was refluxed for 7 hours and then fractionally distilled giving the amino-ester (IX (*b*)) (13·4 g.) b.pt. 142° to 144° C./20 mm., equiv. wt. 225 (calc. 227). It gave a *picrate*, yellow needles from ethanol, m.pt. 63° to 64° C. Found: C, 49·4; H, 5·9; N, 12·4. $C_{19}H_{28}O_9N_4$ requires C, 50·0; H, 6·1; N, 12·3 per cent.

(*c*) (*IX* (*c*)). A mixture of 4-methylpiperidine (14·2 g.) and ethyl crotonate (16·4 g.) was refluxed for 7 hours and then fractionally distilled giving the amino-ester (IX (*c*)) (21 g.) b.pt. 122° to 126° C./20 mm. It gave a *picrate*, yellow needles from ethanol, m.pt. 111° to 112° C. Found: C, 49·1; H, 5·8; N, 12·9 per cent; equiv. wt. 439. $C_{18}H_{26}O_9N_4$ requires C, 48·9; H, 5·9; N, 12·7 per cent.; equiv. wt. 436.

(*d*) (*IX* (*d*)). A mixture of 3-methylpiperidine (18·5 g.) and ethyl crotonate (11·4 g.) was refluxed for 9 hours and then fractionally distilled to give the amino-ester (IX (*d*)) (17·8 g.) b.pt. 120° to 124° C./20 mm. It gave a *picrate*, yellow needles from ethanol, m.pt. 106° to 107° C. Found: C, 49·0; H, 5·7; N, 13·1 per cent; equiv. wt. 438. $C_{18}H_{26}O_9N_4$ requires C, 48·9; H, 5·9; N, 12·7 per cent.; equiv. wt. 436.

(*e*) (*IX* (*e*)). A mixture of 2-methylpiperidine (27·5 g.) and ethyl crotonate (15·8 g.) was refluxed for 17 hours and then fractionally distilled to give the amino-ester (IX (*e*)) (7·1 g.) b.pt. 128° to 130° C./20 mm. It gave a *picrate*, yellow needles from ethanol, m.pt. 109° to 110° C. Found: C, 48·2; H, 5·8; N, 13·3 per cent.; equiv. wt. 445. $C_{18}H_{26}O_9N_4$ requires C, 48·9; H, 5·9; N, 12·7 per cent.; equiv. wt. 436.

3-Amino-1 : 1-di(2'-thienyl)-butan-1-ols (X)

(*a*) (*X* (*a*)). Thiophen (5 g.) in ether (5 ml.) was added to a stirred solution of phenyl lithium in ether (50 ml.) prepared from lithium (0·85 g.) and bromobenzene (9·4 g.), and the mixture refluxed for 2 hours. The amino-ester (IX (*a*)) (4·3 g.) in ether (5 ml.) was added dropwise to the stirred product, cooled by an acetone-solid CO_2 bath. The mixture, after stirring for half-an-hour at room temperature, was poured on to crushed ice and acidified with glacial acetic acid (8 ml.). The solid which separated was washed with ether, the free base liberated with dilute aqueous ammonia and extracted with ether. After drying (Na_2SO_4) the solvent was removed and the residue distilled under reduced pressure to give an orange-coloured oil (3 g.) b.pt. 190° to 200° C./0·2 mm. which solidified on scratching in the presence of acetone. The solid was crystallised from ethanol to give colourless needles of the *amino-butanol* (X (*a*)) m.pt. 80·5° to 81·5° C. Found: C, 63·8; H, 7·2; N, 4·0 per cent.; equiv. wt. 341. $C_{18}H_{25}ONS_2$ requires C, 64·5; H, 7·5; N, 4·2 per cent.; equiv. wt. 335.

(*b*) (*X* (*b*)). Treatment of the amino-ester (IX (*b*)) (4·5 g.) with thienyllithium, as described above, gave a crude base which distilled at 216 to 230° C./0·1 mm. as a yellow oil (3·7 g.) that crystallised from methanol on standing in the refrigerator. Recrystallisation from the same solvent gave colourless plates of the *amino-butanol* (X (*b*)) m.pt. 43° to 44° C. Found: C, 65·4; H, 7·95; N, 4·2 per cent.; equiv. wt. 348. $C_{19}H_{27}ONS_2$ requires C, 65·3; H, 7·7; N, 4·0 per cent.; equiv. wt. 349.

(*c*) (*X* (*c*), (*d*) *and* (*e*)). Treatment of the amino-esters (IX (*c*), (*d*) and (*e*)) with thienyl lithium, as described above, gave the corresponding crude amino-butanols as solids which were crystallised from ethanol without prior distillation.

The *amino-butanol* (X (*c*)) was obtained as colourless plates m.pt. 89° to 90° C. Found: C, 63·9; H, 7·2; N, 4·0 per cent.; equiv. wt. 339. $C_{18}H_{25}ONS_2$ requires C, 64·5; H, 7·5; N, 4·2 per cent.; equiv. wt. 335.

The *amino-butanol* (X (*d*)) was obtained as colourless needles m.pt. 123° to 125° C. Found: C, 64·3; H, 7·4; N, 4·0 per cent.; equiv. wt. 339. $C_{18}H_{25}ONS_2$ requires C, 64·5; H, 7·5; N, 4·2 per cent.; equiv. wt. 335.

The *amino-butanol* (X (*e*)) was obtained as colourless needles m.pt. 101° to 102° C. Found: C, 63·9; H, 7·25; N, 4·4 per cent.; equiv. wt. 337. $C_{18}H_{25}ONS_2$ requires C, 64·5; H, 7·5; N, 4·2 per cent.; equiv. wt. 335.

3-*Amino*-1 : 1-*di*(2′-*thienyl*)-*but*-1-*enes* (*III*; $R = CH_3$)

Dry hydrogen chloride was passed for 10 minutes through a solution of the amino-alcohol (X (*a*)) (1 g.) in chloroform (5 ml.), the solvent removed under reduced pressure, and the residue in water stirred with charcoal for a few minutes at 60° C. The mixture was filtered, the base liberated with dilute aqueous ammonia and extracted with ether. After drying (Na_2SO_4), the solvent was removed to give 3-hexamethylenimino-1 : 1-di(2′-thienyl)-but-1-ene (0·8 g.) as a yellow oil. It gave a *hydrobromide*, grey-green plates from ether-ethanol, m.pt. 137° to 138° C. Found: C, 54·2; H, 6·0; N, 3·5 per cent.; equiv. wt. 406. $C_{18}H_{24}NS_2Br$ requires C, 54·3; H, 6·0; N, 3·5 per cent.; equiv. wt. 398.

Treatment of the amino-butanols (X (*b*), (*c*), (*d*) and (*e*)) with hydrogen chloride as described above gave the corresponding crude amino-butenes.

3-*Heptamethylenimino*-1 : 1-*di*(2′-*thienyl*)-*but*-1-*ene hydriodide* was obtained as grey-green plates from ether-ethanol, m.pt. 131° to 132° C. (*decomp*). Found: C, 49·4; H, 5·7 per cent.; equiv. wt. 462. $C_{19}H_{26}NS_2I$ requires C, 49·7; H, 5·7 per cent.; equiv. wt. 459.

3-(4-*Methylpiperidino*)-1 : 1-*di*(2′-*thienyl*)-*but*-1-*ene hydrobromide* was obtained as pale buff plates from acetone, m.pt. 148° to 148·5° C. (*decomp*). Found: C, 54·0; H, 5·9; N, 3·5 per cent.; equiv. wt. 402. $C_{18}H_{24}NS_2Br$ requires C, 54·3; H, 6·0; N, 3·5 per cent.; equiv. wt. 398.

3-(3-*Methylpiperidino*)-1 : 1-*di*(2′-*thienyl*)-*but*-1-*ene hydrobromide* was obtained as pale buff needles from ether-ethanol, m.pt. 180° to 181° C. (*decomp*). Found: C, 54·5; H, 5·8; N, 3·4 per cent.; equiv. wt. 399. $C_{18}H_{24}NS_2Br$ requires C, 54·3; H, 6·0; N, 3·5 per cent.; equiv. wt. 398.

3-(2-*Methylpiperidino*)-1 : 1-*di*(2′-*thienyl*)-*but*-1-*ene* was obtained as a

yellow oil. Found: C, 68·1; H, 7·3 per cent.; equiv. wt. 316. $C_{18}H_{23}NS_2$ requires C, 68·1; H, 7·25 per cent.; equiv. wt. 317.

Measurement of dissociation constants

Titrations were carried out in a glass micro-cell with a thick walled capillary tube sealed into the base. This permitted nitrogen to be bubbled up through the solution to stir it, remove other dissolved gases and maintain an inert atmosphere. The cell was immersed in a thermostat at 25° C. $\pm$ 0·1°, and the nitrogen freed from carbon dioxide by passage through 10 per cent. potassium hydroxide in a gas-washing bottle with a sintered glass disc and a trap, both immersed in the thermostat. Doran alkacid and micro saturated calomel electrodes were used in conjunction with a modified Cambridge pH meter, details of which will be published elsewhere. An Agla micrometer syringe was used as a burette.

Quantities of 10 mg. of the free bases or their salts were dissolved in sufficient 0·01N carbon dioxide-free hydrochloric acid to give a total concentration of approximately 0·013M in the region of the titration curve at which calculations were made, and the solutions diluted to 10 ml. with carbon dioxide-free distilled water. Aliquots of 5 ml. were titrated potentiometrically with 0·1N carbonate-free sodium hydroxide prepared by dilution from 18N solution followed by passage through a column of ion exchange resin IRA–400 as described by Davies and Nancollas[29]. Aliquot quantities were used since it was necessary to know the exact concentration of base for the purpose of the calculations.

pK'a values were calculated from the equation:—

$$pK'a = pH + \log \frac{Ca - Cb}{C - (Ca - Cb)}$$

where C, Ca and Cb are the total concentrations of organic base, strong acid and strong base respectively. Two points on each of at least two titration curves were used for the calculations. The reproducibility of the results was within $\pm$0·05 pK units.

The dissociation constants for various thiambutene-type compounds are recorded in Table III. Compounds 28 to 32 were prepared in the present work. Compounds 18, 23 and 27 were kindly provided by Dr. H. T. Openshaw and Mr. A. F. Green. Compound 33 has been described previously[30].

Pharmacological testing

The analgesic activity of compounds 28–32 (see Table II) was tested in rats using heat and pressure methods. The piperidino compound (27) (see Green[13]) was used as a standard for comparison. The results are recorded in Table II (activities expressed against morphine to allow comparison with other results quoted in this Table).

SUMMARY

1. The analgesic activity of several series of methadone type compounds is shown to decrease upon the increase in the "effective width" of the basic group.

2. The steric requirements about the anionic site of the analgesic receptor are discussed.

3. Dissociation constants and analgesic activities of a series of thiambutene-type compounds are presented. Evidence is given for their probable conformation in aqueous solution. Their activities are shown to decrease with increase in the "effective width" of their basic groups.

4. The preparation of certain thiambutene-type analgesics is described.

5. A method is described for the determination of dissociation constants in aqueous solution of sparingly water soluble bases.

The authors wish to express their thanks to Dr. H. T. Openshaw and Mr. A. F. Green (Wellcome Research Laboratories) for supplying Compounds 18, 23 and 27 and to Mr. Green for carrying out the pharmacological testing. They also thank Mr. C. Morton for advice in the measurement of dissociation constants.

REFERENCES

1. Beckett, *J. Pharm. Pharmacol.*, 1956, **8**, 848.
2. Clarke, Pessolano, Weijlard and Pfister, 3rd., *J. Amer. chem. Soc.*, 1953, **75**, 4963.
3. Green, Ruffell and Walton, *J. Pharm. Pharmacol.*, 1954, **6**, 390.
4. Braenden, Eddy and Halbach, *Bull. World Hlth. Org.*, 1955, **13**, 937.
5. Beckett and Casy, *J. Pharm. Pharmacol.*, 1954, **6**, 986.
6. Ofner and Walton, *J. chem. Soc.*, 1950, 2158.
7. Kleiderer, Rice, Conquest and Williams, Report No. P.B. 981, Office of Publication Board, Dept. of Commerce, Wash.
8. Bockmühl and Ehrhart, *Liebigs Ann.*, 1948, **561**, 52.
9. Attenburrow, Elks, Hems and Speyers, *J. chem. Soc.*, 1949, 510.
10. Blicke and Tsao, *J. Amer. chem. Soc.*, 1954, **76**, 2203.
11. Dupré, Elks, Hems, Speyer and Evans, *J. chem. Soc.*, 1954, 500.
12. Morrison and Rinderknecht, *ibid.*, 1950, 1478.
13. Green, *Brit. J. Pharmacol.*, 1953, **8**, 2.
14. Beckett, Casy and Harper, *J. Pharm. Pharmacol.*, 1956, **8**, 874.
15. Adamson, *J. chem. Soc.*, 1950, 885.
16. Adamson and Green, *Nature, Lond.*, 1950, **165**, 122.
17. Costa and Bonnycastle, *J. Pharmacol.*, 1955, **113**, 12.
18. Flintan and Keele, *ibid.*, 1954, **110**, 18.
19. Turner and Harris, *Organic Chemistry*, Longmans, Green and Co., London, 1952, p. 692.
20. Gero, *Science*, 1954, **119**, 112.
21. Beckett and Phillips, unpublished work.
22. Petersen, *Acta pharm. tox., Kbh.*, 1953, **9**, 322.
23. Ruzicka, Kobelt, Häfliger and Prelog., *Helv. chim. Acta.*, 1949, **32**, 544.
24. Wallach, *Liebigs Ann.*, 1899, **309**, 19.
25. Hall, *J. phys. Chem.*, 1956, **60**, 63.
26. Beckett and Tinley, *Titration in Non-Aqueous Solvents*, The British Drug Houses Ltd., Poole, England.
27. Pifer and Wollish, *J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 609.
28. Ruzicka, Goldberg, Hurbin and Boeckenoogen, *Helv. chim. Acta.*, 1933, **16**, 1335.
29. Davies and Nancollas, *Nature, Lond.*, 1950, **165**, 237.
30. Beckett and Casy, *J. chem. Soc.*, 1955, 900.