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STUDIES OF 3-O-ACYL DERIVATIVES OF NALOXONE AS ITS POTENTIAL PRODRUGS

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An efficient method has been proposed for the preparation of a series of 3-O-acyl derivatives of naloxone. The features of the steric structure and NMR spectra are discussed. Pharmaceutical investigation has shown the promise within the synthesized compounds of creating opiate receptor antagonist compounds with prolonged action.

Keywords: 3-hydroxymorphinans, opiate receptor antagonists, naloxone, prodrugs, acylation, X-ray structural analysis.

It is known that substances of the "pure" opiate receptor blocker class such as naloxone and naltrexone efficiently suppress the pharmacological effects of the opiate series narcotics: morphine, heroin, methadone, etc. Hence at this time they are widely used in acute opioid narcotic intoxication and also against relapse in the treatment of opioid addiction [1-4] and alcoholism [5-7].

Assigning these preparations for opioid addiction involves a complete exclusion of the possibility of building up opioid intoxication in narcotic illness usage. Hence a possible narcotic excess in the area of action of opiate receptor blockers should not lead to the onset of illness relapse. The therapeutic effect of opiate receptor blockers in relation to alcoholism is not so complete. Hence they are not in place for avoidance of alcohol dependence. In patients there are generally observed disturbances to walking and speech and changes to thinking and reaction times etc. In terms of opiate receptor blockers they block only the emotional reaction to the alcohol and the euphoric component of its activity, i.e. just that for which the alcohol is used. As a result, the use of alcohol after the dose of opiate receptor blockers significantly loses purpose.

None the less, despite the high efficiency found in the current arsenal of opiate receptor blockers they are basically used as preparations of rapid relief. In fact their widespread use as antirelapse agents hides a deficiency in prolonged action. The desire of a narcotic patient to use the corresponding medication for this particular group of illnesses fluctuates greatly. Hence investigation of the creation of opiate receptor blockers with prolonged action assumes special importance at this time.

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In our view one of the simplest solutions to the problems described above may be a bio-reversible modification to create a prodrug based on one of the well known opiate receptor blockers, e.g. naloxone. The change in several physicochemical properties of this medicine is sure to reflect on its pharmaceutical and/or its pharmacokinetic parameters. If these changes can be carried out in the desired direction the prodrug obtained in this way can be gradually converted in the living organism to the starting naloxone, proving the same characteristic therapeutic effect but extending its course.

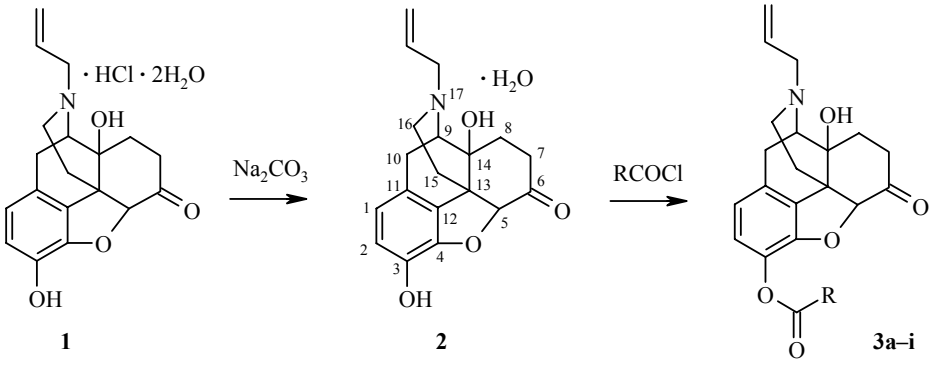
Analysis of the chemical structure of naloxone **1** shows that perhaps the most convenient method for its chemical modification to a prodrug is conversion of one of the hydroxyl groups to an ester. Several factors provide the basis for this conclusion. Primarily, this is the absence of overload by this type of prodrug on human organism enzyme systems since esterases critical for biotransformation of esters relate to a number of the most widely distributed enzymes in all organs and tissues and are in significant amounts [8].

On the other hand unlimited possibilities of a change in the structure of the acid part of the ester allows in broad terms a variation of such important properties of future prodrugs as their solubility and rate of release of the starting medicine and this opens up the possibility of achieving the most optimal result.

One final, extremely significant merit of the proposed method of possible modification of naloxone to a prodrug ester is its simplicity and that it can be carried out by simple and readily carried out chemical reactions.

Treatment of an aqueous solution of naloxone **1** with sodium carbonate gives the naloxone base hydrate **2**. X-ray analytical investigation of the structure of this compound (see Fig. 1 and Tables 1 and 2) shows that in its carcass polycyclic system the cyclohexanone ring is in a *chair* conformation (folding parameters [9]: $S = 0.97$, $\theta = 11.9^{\circ}$, $\psi = 15.7^{\circ}$). The deviations of atoms C(2) and C(5) from the best mean square plane for the remaining ring atoms are -0.68 and 0.41 Å respectively. The cyclohexene ring has a *sofa* type conformation (folding parameters: $S = 0.80$, $\theta = 35.3^{\circ}$, $\psi = 1.6^{\circ}$) in which atom C(1) deviates from the mean plane of the remaining ring atoms by -0.73 Å. The piperidine fragment occurs in a *chair* conformation (folding parameters: $S = 1.24$, $\theta = 5.9^{\circ}$, $\psi = 6.6^{\circ}$). The deviations of atoms C(14) and C(15) from the best mean plane for the remaining atoms are -0.82 and 0.65 Å respectively.

Scheme 1



1 **2** **3a–i**

3 a R = C_8H_{17}, **b** R = C_9H_{19}, **c** R = $(CH_2)_8CH{=}CH_2$, **d** R = $C_{10}H_{21}$, **e** R = $C_{11}H_{23}$, **f** R = $C_{12}H_{25}$, **g** R = $C_{13}H_{27}$, **h** R = $C_{14}H_{29}$, **i** R = $C_{15}H_{31}$

The dihydrofuran ring is found in an *envelope* type conformation, the deviation of atom C(5) from the mean plane of the remaining ring atoms being -0.52 Å.

The nitrogen atom has a pyramidal conformation, the sum of the valence angles centered on it being 338º. The allyl substituent has a cisoid conformation (torsional angle N(1)–C(17)–C(18)–C(19) 12.5(3)º) and occurs in an equatorial position twisted virtually perpendicularly to the C(16)–N(1) bonds of the piperidine ring (torsional angles

C(17)–N(1)–C(16)–C(15) -170.3(1)º and C(16)–N(1)–C(17)–C(18) 77.8(2)º). Repulsion between the atoms of the piperidine and cyclohexene rings and the allyl group [shortened intramolecular contacts H(14)···H(17a) 2.18 (sum of van der Waal radii 2.34 [10]), H(13b)···C(17) 2.80 (2.87), H(16b)···C(18) 2.78 (2.87), and H(16b)···C(19) 2.85 Å (2.87 Å)] facilitates such a position for this fragment.

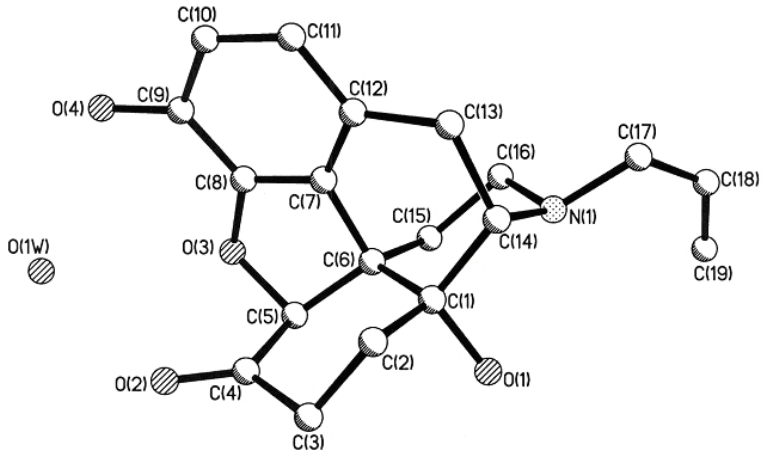


Fig. 1. Structure of the naloxone base hydrate **2** with atomic numbering.

The naloxone base hydrate **2** molecule is sterically hindered to a significant degree as indicated by the shortened intramolecular contacts H(2a)···C(7) 2.71 (2.87), H(2a)···C(12) 2.83 (2.87), H(2a)···C(13) 2.80 (2.87), H(16a)···C(7) 2.79 (2.87), H(16a)···C(12) 2.73 (2.87), and H(16a)···C(13) 2.70 Å (2.87 Å). The hydroxyl group at atom C(1) forms an intramolecular hydrogen bond with the piperidine ring nitrogen atom O(1)–H(1O)···N(1) (H···N 1.99 Å , O–H···N 128º).

In the crystal the molecules of compound **2** are mutually linked through a bridging water molecule *via* intermolecular hydrogen bonds: O(4)–H(4O)···O(1w) (H···O 1.90 Å, O–H···O 170º), O(1w)–H(1wa)···O(2) (H···O 2.14 Å, O–H···O 168º), and O(1w)–H(1wb)···O (1)' (0.5+*x*, -0.5-*y*, -*z*) H···O 1.94 Å, O–H···O 178º. Shortened intermolecular contacts are also seen in the crystal H(3b)···C(8)' (*x*-1, *y*, *z*), 2.82 (2.87) and H(3b)···C(9)' (*x*-1, *y*, *z*) 2.76 Å (2.87 Å).

TABLE 1. Bond Lengths (*l*) in the Naloxone Base Hydrate **2** Structure

Bond	*l*, Å	Bond	*l*, Å
N(1)–C(17)	1.466(2)	N(1)–C(16)	1.473(2)
N(1)–C(14)	1.482(2)	O(1)–C(1)	1.437(2)
O(2)–C(4)	1.209(2)	O(3)–C(8)	1.387(2)
O(3)–C(5)	1.462(2)	O(4)–C(9)	1.363(2)
C(1)–C(2)	1.516(2)	C(1)–C(6)	1.539(2)
C(1)–C(14)	1.549(2)	C(2)–C(3)	1.538(2)
C(3)–C(4)	1.494(2)	C(4)–C(5)	1.534(2)
C(5)–C(6)	1.544(2)	C(6)–C(7)	1.510(2)
C(6)–C(15)	1.546(2)	C(7)–C(12)	1.366(2)
C(7)–C(8)	1.380(2)	C(8)–C(9)	1.387(2)
C(9)–C(10)	1.398(3)	C(10)–C(11)	1.385(3)
C(11)–C(12)	1.404(2)	C(12)–C(13)	1.511(2)
C(13)–C(14)	1.552(2)	C(15)–C(16)	1.531(2)
C(17)–C(18)	1.492(3)	C(18)–C(19)	1.301(3)

TABLE 2. Valence Angles (ω) in the Naloxone Base Hydrate **2** Structure

Angle	ω, deg	Angle	ω, deg
C(17)–N(1)–C(16)	112.6(1)	C(17)–N(1)–C(14)	113.7(1)
C(16)–N(1)–C(14)	111.7(1)	C(8)–O(3)–C(5)	103.8(1)
O(1)–C(1)–C(2)	106.8(1)	O(1)–C(1)–C(6)	110.8(1)
C(2)–C(1)–C(6)	111.9(1)	O(1)–C(1)–C(14)	107.3(1)
C(2)–C(1)–C(14)	113.9(1)	C(6)–C(1)–C(14)	106.1(1)
C(1)–C(2)–C(3)	110.7(1)	C(4)–C(3)–C(2)	111.3(1)
O(2)–C(4)–C(3)	122.7(1)	O(2)–C(4)–C(5)	120.2(1)
C(3)–C(4)–C(5)	117.1(1)	O(3)–C(5)–C(4)	107.2(1)
O(3)–C(5)–C(6)	105.1(1)	C(4)–C(5)–C(6)	113.5(1)
C(7)–C(6)–C(1)	108.2(1)	C(7)–C(6)–C(5)	98.2(1)
C(1)–C(6)–C(5)	117.7(1)	C(7)–C(6)–C(15)	110.0(1)
C(1)–C(6)–C(15)	109.7(1)	C(5)–C(6)–C(15)	112.2(1)
C(12)–C(7)–C(8)	124.3(1)	C(12)–C(7)–C(6)	127.0(1)
C(8)–C(7)–C(6)	108.7(1)	C(7)–C(8)–O(3)	112.0(1)
C(7)–C(8)–C(9)	120.8(1)	O(3)–C(8)–C(9)	127.1(1)
O(4)–C(9)–C(8)	125.0(2)	O(4)–C(9)–C(10)	119.5(2)
C(8)–C(9)–C(10)	115.5(1)	C(11)–C(10)–C(9)	123.2(2)
C(10)–C(11)–C(12)	120.5(2)	C(7)–C(12)–C(11)	115.6(1)
C(7)–C(12)–C(13)	118.3(1)	C(11)–C(12)–C(13)	125.9(1)
C(12)–C(13)–C(14)	114.7(1)	N(1)–C(14)–C(1)	104.3(1)
N(1)–C(14)–C(13)	116.1(1)	C(1)–C(14)–C(13)	114.8(1)
C(16)–C(15)–C(6)	110.6(1)	N(1)–C(16)–C(15)	110.5(1)
N(1)–C(17)–C(18)	113.7(2)	C(19)–C(18)–C(17)	126.9(2)

This X-ray analysis shows that the tertiary alcohol hydroxyl group in the naloxone hydrate base **2** molecule proves to be significantly sterically hindered. Because of the steric features of the molecule approach to it is hindered and thus ready acylation is hardly possible. On the other hand the phenolic hydroxyl experiences no kind of steric hindrance and thus acylation will primarily occur at this position. In fact there are many examples of the synthesis of acyl derivatives of 3-hydroxymorphinans including naloxone and naltrexone [11-13] which confirm this experimentally.

Creation in the organism of a depot (and in fact extending the action of many medicines on this basis) is most conveniently carried out for opiate receptors blockers using injected solutions [14-17]. With this feature in mind we have used a series of higher carboxylic acids for the acylation of naloxone base **2**, starting with nonanoic and finishing with the hexadecanoic. This choice is made because of their high lipophilicity and thus solubility in fats. It is eascest way to create corresponding pharmaceutical forms, for example oil injection solutions on the basis of just these esters. In addition, such"heavy" esters *in vivo* generally show less tendency towards hydrolysis and on this account show markedly greater prolongation of activity than their homologs with shorter acids.

According to known methods [11-13], 3-hydroxymorphinans are acylated both by the organic acids themselves in the presence of carbodiimides as dehydrating agents and of 4-dimethylaminopyridine type catalysts and also *via* their different derivatives: isocyanates, acid chlorides (sulfochlorides), anhydrides, or mixed anhydrides with the need to combine the free acids evolved using alkali metals, their carbonates, bicarbonates, or tertiary amines. In all cases the reaction is carried out in anhydrous, aprotic solvents for 0.5-24 h over a wide temperature range and sometimes under a nitrogen atmosphere.

As indicated above, when naloxone **1** is treated with sodium carbonate in water salt formation with phenolic hydroxyl does not occur. The naloxone base hydrate **2** is readily extracted with many organic solvents. This also served as the base for the method we have proposed for the acylation of naloxone **1** using acid

TABLE 3. Characteristics of the 3-O-Acyl Derivatives of Naloxone **3a-i**

Compound	Empirical formula	Found, % / Calculated, %			mp, °C*	Yield, %	Prolongation of opioid intoxication action in the test (pain sensitivity), days
		C	H	N			
3a	$C_{28}H_{37}NO_5$	72.04 / 71.92	8.09 / 7.98	2.93 / 3.00	66-68	91	5 (6)
3b	$C_{29}H_{39}NO_5$	72.41 / 72.32	8.27 / 8.16	2.85 / 2.91	72-74	93	8 (9)
3c	$C_{30}H_{39}NO_5$	73.10 / 72.99	8.05 / 7.96	2.92 / 2.84	64-66	98	8 (9)
3d	$C_{30}H_{41}NO_5$	72.79 / 72.70	8.45 / 8.34	2.76 / 2.83	69-71	93	8 (8)
3e	$C_{31}H_{43}NO_5$	73.17 / 73.05	8.61 / 8.50	2.69 / 2.75	68-70	95	17 (>19)
3f	$C_{32}H_{45}NO_5$	73.30 / 73.39	8.72 / 8.66	2.71 / 2.67	58-60	98	19 (>19)
3g	$C_{33}H_{47}NO_5$	73.63 / 73.71	8.75 / 8.81	2.69 / 2.60	57-59	97	18 (>19)
3h	$C_{34}H_{49}NO_5$	73.90 / 74.01	8.87 / 8.95	2.45 / 2.54	62-64	99	12 (15)
3i	$C_{35}H_{51}NO_5$	74.24 / 74.30	8.96 / 9.09	2.39 / 2.48	61-63	94	11 (12)

* Solvent: ethanol (compounds **3a,b**) and hexane (compounds **3c-i**).

chlorides in a two-phase water–organic system in the presence of the cheap, available and nontoxic sodium carbonate. In principle, other water immiscible solvent can be used which do not give stable solvent emulsions. However, preference is given to methylene chloride or chloroform, use of which significantly eases the separation of the final products. Overall, this method allows the preparation of the target 3-O-acyl naloxone derivatives **3a-i** in very high yields and guarantees their purity sufficient for the creation of injection pharmaceutical forms – according to HPLC data. Content of the base materials is at least 99.6% in each sample.

All of the 3-O-acyl naloxone derivatives obtained **3a-i** are colorless, crystalline materials with relatively low melting points (Table 3) and are readily soluble in the majority of organic solvents but virtually insoluble in water.

The uncommon structure of the 3-O-acyl naloxone derivatives **3a-i** is certainly of significant interest for NMR spectroscopy but investigation is not simple for a known reason. Hence we have initially made a detailed analysis of the NMR spectra of the somewhat simpler naloxone base hydrate structure **2**. The molecule of compound contains a large number of nonequivalent protons and an assignment of their signals on the basis of the proton spectrum alone cannot be made. Hence we have measured its ^{1}H and ^{13}C NMR spectra and additionally carried out homonuclear (COSY, NOESY) and heteronuclear ^{1}H–^{13}C correlation experiments. All of these were carried out for a solution of compound **2** in DMSO-d_6 in order to get a high concentration solution and determine the position in the spectrum of active proton signals. However, the spectrum in this solvent proved somewhat broad such that an exact determination of the spin-spin coupling values for the compound which were important for clarifying its stereochemical features became impossible. For this reason the spectrum of the naloxone base hydrate **2** was also measured in deuterochloroform in order to analyze the spin multiplets for the aliphatic protons. Although the signals for the hydroxyl group protons were not seen in this solvent all of the remaining signals are significantly sharper and allow much more exact spin-spin couplings to be obtained.

Both in the proton and carbon spectrum of the naloxone base hydrate **2** there are several signals whose assignments are unambiguous. In the proton spectrum these are the signals for the allyl substituent, the doublets for the aromatic protons H-1 and H-2, and also the singlet for proton H-5 on the nodal carbon atom between the furan and cyclohexanone rings. As reference parameters in the ^{13}C NMR spectra the carbonyl atom C(6) and also the nodal carbon atoms C(5), C(9), and C(14), whose positions are determined by the presence of electronegative substituents in the molecule, can be used. The presence of heteronuclear correlations with these

signals permits the assignment of other signals in the proton and carbon spectra. For convenience we have gathered the homonuclear and heteronuclear correlations found for the protons signals of compound **2** measured in DMSO-d_6 in Table 4.

The assignment of the ^1H and ^{13}C NMR signals in the cyclohexanone fragment of the molecule is most conveniently made from the presence of correlation of the signals of all of the protons in this ring with the carbonyl carbon atom found at 209.4 ppm. As seen from the data in Table 4 there are two methylene groups (7- and 8-CH_2) and one methine proton (H-5) whose signals correlate with the carbonyl carbon atom. Hence in this fragment there are assigned methylene groups with the ^{13}C NMR shifts 31.8 and 36.5 ppm which are linked to the geminal protons with chemical shifts of 1.71 and 1.43 ppm and also 2.88 and 2.06 ppm respectively. The geminal pair with proton signals at lower field correspond to the methylene group placed next to the carbonyl. This is supported by the presence of a correlation of the signal for one of these methylene group protons at 2.06 ppm with the nodal atom C(5) at 90.0 ppm. The single methine proton which can give a correlation with the carbonyl carbon atom in the HMBC spectrum is H-5 proton at 4.75 ppm which is found at the nodal C(5) atom placed between the dihydrofuran and cyclohexanone rings.

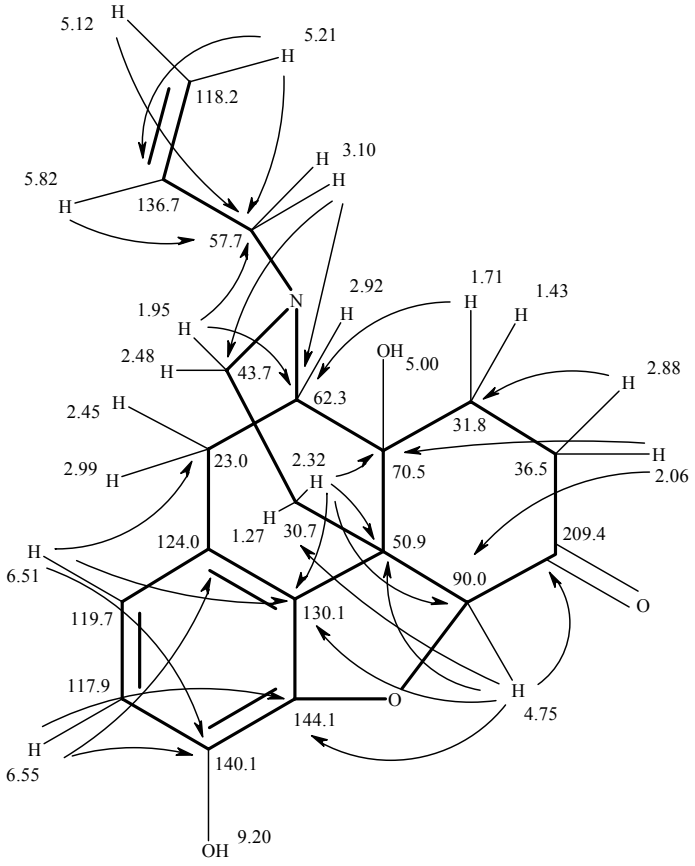
The ^1H and ^{13}C signals for the 15- and 16-CH_2 groups in the piperidine ring can be assigned on the basis of the correlation of the signals of their carbon atoms with the allyl substituent proton signals and the H-5 proton signal with the chemical shift of 4.75 ppm which has been mentioned above. Interpretation of the

TABLE 4. Homonuclear and Heteronuclear ^1H–^{13}C Correlations Observed in the Naloxone Base Hydrate **2**

δ, ppm				
^1H	HMQC	HMBC	COSY	NOESY
9.20	—	—	—	5.00 (–), 3.33 (–)
6.55	117.9	144.1, 140.1, 124.0	6.51	6.51, 3.33
6.51	119.7	50.9, 23.0, 140.1, 130.1, 117.9	6.55	6.55, 2.99, 2.48
5.82	136.7	57.7	5.21, 5.12, 3.10	3.10, 2.92, 5.21, 5.12
5.21	118.2	57.7, 136.7	5.12, 3.10	3.10, 5.82
5.12	118.2	57.7	5.21, 3.10	5.82, 5.21
5.00	—	—	—	9.20 (–)
4.75	90.0	209.4, 50.9, 30.7, 144.1, 130.1	—	2.92, 2.32, 1.27
3.10	57.7	62.3, 43.7, 136.7, 118.2	5.12, 5.21	2.99, 2.92, 2.48, 1.95
2.99	23.0	70.5, 62.3, 140.1, 130.1, 124.0, 119.7, 117.9	2.45	2.45, 1.95
2.92	62.3	90.0, 70.5, 57.7, 50.9, 43.7, 23.0	—	3.10, 2.48, 2.06, 1.71, 4.75
2.88	36.5	31.8	2.06, 1.43	3.33, 2.06
2.48	43.7	57.7, 50.9, 30.7, 124.0	2.32, 1.95	2.32, 1.95
2.45	23.0	209.4, 62.3, 144.1, 140.1, 130.1, 124.0, 119.7, 117.9	2.99	2.99, 1.43
2.32	30.7	90.0, 70.5, 50.9, 43.7, 130.1	2.45, 1.95, 1.27	1.27, 1.95, 4.75
2.06	36.5	209.4, 90.0, 70.5	2.88, 1.71, 1.43	2.92, 1.71, 1.43
1.95	43.7	62.3, 57.7, 30.7	2.32, 2.48, 1.27	1.27, 2.48, 2.32
1.71	31.8	209.4, 70.5, 62.3, 50.9, 36.5	1.43, 2.06, 2.22	1.43, 2.92
1.43	31.8	209.4, 36.5	1.71, 2.06, 2.88	1.71, 2.06, 2.45
1.27	30.7	70.5, 50.9, 43.7	1.95, 2.32	2.32, 1.95, 4.75

remaining signals did not present a difficulty. The signal assignments found are shown in Scheme 2 with the most important HMBC correlations which served as the basis for these assignments. The signals for the protonated carbon atoms are assigned from their HMQC correlations (Table 4).

Scheme 2

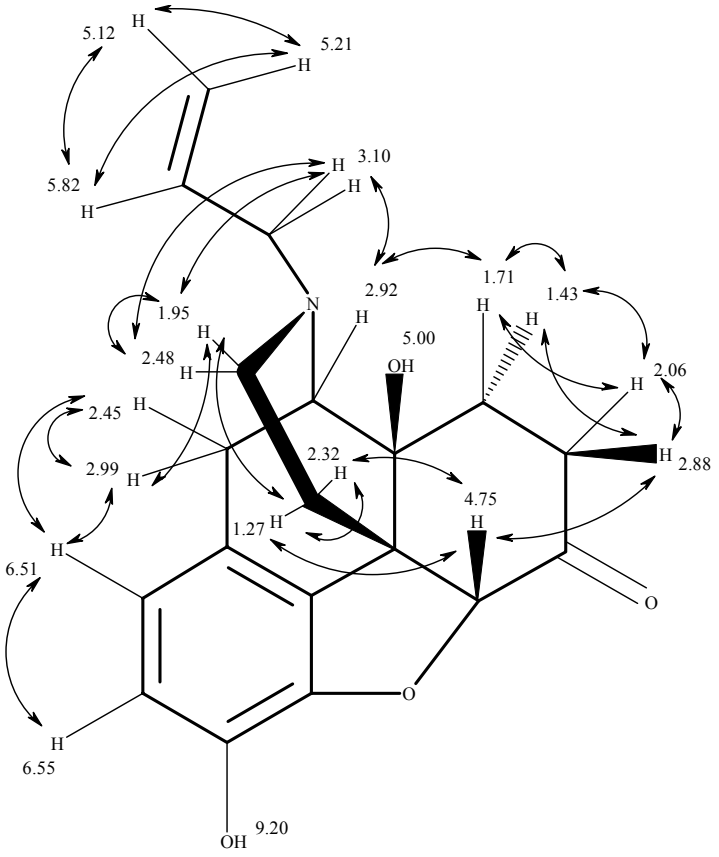


The assignments of signals in the proton spectra also allowed the determination of the stereochemical features of the naloxone base hydrate molecule **2** in solution. With this aim we have analyzed the COSY and NOESY spectra. The NOESY correlations are given below in Scheme 3. The presence of a correlation between the signal for proton H-5 at 4.75 ppm with the 15-CH_2 group protons indicates the spatial proximity of these protons. Hence proton H-5 is axial and directed towards the piperidine ring side as shown in the Scheme. The presence of the correlation for the H-5 proton with the signal for one of the 7-CH_2 methylene group protons at 2.88 ppm infers that this proton is also axial. Hence its geminal pair partner at 2.06 ppm has an equatorial orientation. In the 8-CH_2 geminal pair of methylene protons the equatorial proton has a chemical shift of 1.71 ppm since it gives a strong correlation in the NOESY spectrum with the signal for the H-9 proton at 2.92 ppm, the equatorial orientation of which is determined by the structure of the molecule. It follows from this that the proton of this geminal group with a chemical shift of 1.43 ppm is axial and has an antiperiplanar orientation relative to other axial protons associated with this ring.

Further stereochemical conclusions can be drawn from comparison of the geminal and vicinal spin-spin coupling values for spin-related proton signals. As is known, resolution of similar problems can only be made when working with exact spin-spin values. We obtained these from the ^{1}H NMR spectra recorded in deuterochloroform (see Experimental). Thus for the 7- and 8-CH_2 groups protons in the cyclohexanone ring the

angle between neighboring axial protons according the steric model is 177º and so, for a vicinal interaction, the spin-spin coupling must be greatest (according to the Karplus relationship [18]). It follows that the signal with a chemical shift of 1.43 ppm corresponds to the axial proton since its geminal and vicinal coupling constants are almost identical at 14.4 Hz. Similarly, in the piperidine ring, the axial protons correspond to the signals at 2.32 and 2.48 ppm because their vicinal couplings are J = 12.4 Hz. In the cyclohexene fragment a

Scheme 3



vicinal coupling is not observed for the signal having a chemical shift of 2.99 ppm with the H-9 proton. This infers that the dihedral angle between these protons is close to 90º. According to the steric model such a situation has to be observed for the 10-CH_2 axial methylene proton and the signal with the chemical shift of 2.99 ppm is assigned to it.

Based on this investigation of the structure of the naloxone base hydrate **2** using the NMR method the problem of interpreting the ^{1}H NMR spectra of the O-allyl derivatives **3a-i** is markedly simplified and virtually reduced to separating out the signals for the protons of the acid fragments (Table 5).

Study of the specific activity of the synthesized 3-O-acyl derivatives of naloxone **3a-i** was carried out for injection pharmaceutical forms with ethyl oleate as solvent and used nonpedigree, white rats.

Through carrying out the biological investigations it was found that the 3-O-acyl derivatives of naloxone **3a-i** indeed show opiate receptor blocking properties and can, to one degree or another, inhibit all of the basic pharmacological effects brought about by morphine in rats, i.e. cataleptogenesis, inhibition of respiration, increased pain threshold etc. As was expected, length of the specific opioprotective action of esters **3a-i** proved different and was determined by the structure of the modified acid fragment (Table 3). Thus, for example, the maximum effect for all of the compounds studied by us occurred in the naloxone ester of with

TABLE 5. ^{1}H NMR Spectra of the 3-O-Acyl Naloxone Derivatives **3a-i**

Com-pound	Chemical shifts (DMSO-d_6), δ, ppm (*J*, Hz)
3a	6.80 (1H, d, ^{3}J = 8.2, H-2); 6.70 (1H, d, ^{3}J = 8.2, H-1); 5.84 (1H, m, β-CH-allyl); 5.22 (1H, d, ^{3}J = 17.8, γ-CH-allyl, *trans*); 5.13 (1H, d, ^{3}J = 10.7, γ-CH-allyl, *cis*); 5.05 (1H, s, 14-OH); 4.87 (1H, s, H-5); 3.12 (2H, m, NCH_2); 3.00 (1H, d, ^{3}J = 15.8, H-10*a*); 2.91 (1H, m, H-9); 2.87 (1H, m, H-7*a*); 2.53 (4H, m, H-10*e* + H-16*e* + $COCH_2$); 2.32 (1H, m, H-15*a*); 2.06 (1H, m, H-7*e*); 1.95 (1H, m, H-16*a*); 1.74 (1H, m, H-8*e*); 1.59 (2H, m, $COCH_2C\underline{H}_2$); 1.43 (1H, m, H-8*a*); 1.23 (11H, m, H-15*e* + $(C\underline{H}_2)_5CH_3$); 0.83 (3H, t, ^{3}J = 6.7, CH_3)
3b	6.80 (1H, d, ^{3}J = 8.2, H-2); 6.71 (1H, d, ^{3}J = 8.2, H-1); 5.84 (1H, m, β-CH-allyl); 5.22 (1H, d, ^{3}J = 17.8, γ-CH-allyl, *trans*); 5.12 (1H, d, ^{3}J = 10.5, γ-CH-allyl, *cis*); 5.05 (1H, s, 14-OH); 4.87 (1H, s, H-5); 3.11 (2H, m, NCH_2); 3.00 (1H, d, ^{3}J = 15.6, H-10*a*); 2.92 (1H, m, H-9); 2.87 (1H, m, H-7*a*); 2.54 (4H, m, H-10*e* + H-16*e* + $COCH_2$); 2.32 (1H, m, 15-H*a*); 2.05 (1H, m, H-7*e*); 1.95 (1H, m, H-16*a*); 1.74 (1H, m, H-8*e*); 1.59 (2H, m, $COCH_2C\underline{H}_2$); 1.44 (1H, m, H-8*a*); 1.23 (13H, m, H-15*e* + $(C\underline{H}_2)_6CH_3$); 0.82 (3H, t, ^{3}J = 6.6, CH_3)
3c	6.80 (1H, d, ^{3}J = 8.2, H-2); 6.70 (1H, d, ^{3}J = 8.2, H-1); 5.79 (2H, m, β-CH-allyl + $(CH_2)_8C\underline{H}{=}CH_2$); 5.22 (1H, d, ^{3}J = 17.8, γ-CH-allyl, *trans*); 5.13 (1H, d, ^{3}J = 10.5, γ-CH-allyl, *cis*); 5.05 (1H, s, 14-OH); 4.97 (1H, d, ^{3}J = 16.7, $(CH_2)_8CH{=}C\underline{H}_2$, *trans*); 4.90 (1H, d, ^{3}J = 10.2, $(CH_2)_8CH{=}C\underline{H}_2$, *cis*); 4.86 (1H, s, H-5); 3.12 (2H, m, NCH_2); 2.99 (1H, d, ^{3}J = 15.5, H-10*a*); 2.93 (1H, m, H-9); 2.86 (1H, m, H-7*a*); 2.54 (4H, m, H-10*e* + H-16*e* + $COCH_2$); 2.32 (1H, m, 15-H*a*); 2.06 (1H, m, H-7*e*); 1.97 (1H, m, H-16*a*); 1.74 (1H, m, H-8*e*); 1.60 (2H, m, $COCH_2C\underline{H}_2$); 1.40 (1H, m, H-8*a*); 1.26 (13H, m, H-15*e* + $(C\underline{H}_2)_6CH{=}CH_2$)
3d	6.80 (1H, d, ^{3}J = 8.2, H-2); 6.70 (1H, d, ^{3}J = 8.2, H-1); 5.85 (1H, m, β-CH-allyl); 5.23 (1H, d, ^{3}J = 17.7, γ-CH-allyl, *trans*); 5.12 (1H, d, ^{3}J = 10.6, γ-CH-allyl, *cis*); 5.05 (1H, s, 14-OH); 4.88 (1H, s, H-5); 3.12 (2H, m, NCH_2); 3.01 (1H, d, ^{3}J = 15.8, H-10*a*); 2.94 (1H, m, H-9); 2.87 (1H, m, H-7*a*); 2.55 (4H, m, H-10*e* + H-16*e* + $COCH_2$); 2.32 (1H, m, 15-H*a*); 2.06 (1H, m, H-7*e*); 1.95 (1H, m, H-16*a*); 1.74 (1H, m, H-8*e*); 1.57 (2H, m, $COCH_2C\underline{H}_2$); 1.43 (1H, m, H-8*a*); 1.25 (15H, m, H-15*e* + $(C\underline{H}_2)_7CH_3$); 0.85 (3H, t, ^{3}J = 6.6, CH_3)
3e	6.80 (1H, d, ^{3}J = 8.2, H-2); 6.70 (1H, d, ^{3}J = 8.2, H-1); 5.84 (1H, m, β-CH-allyl); 5.22 (1H, d, ^{3}J = 17.4, γ-CH-allyl, *trans*); 5.13 (1H, d, ^{3}J = 10.3, γ-CH-allyl, *cis*); 5.04 (1H, s, 14-OH); 4.87 (1H, s, H-5); 3.12 (2H, m, NCH_2); 3.00 (1H, d, ^{3}J = 15.5, H-10*a*); 2.93 (1H, m, H-9); 2.87 (1H, m, H-7*a*); 2.55 (4H, m, H-10*e* + H-16*e* + $COCH_2$); 2.32 (1H, m, 15-H*a*); 2.06 (1H, m, H-7*e*); 1.93 (1H, m, H-16*a*); 1.74 (1H, m, H-8*e*) 1.58 (2H, m, $COCH_2C\underline{H}_2$); 1.40 (1H, m, H-8*a*); 1.22 (17H, m, H-15*e* + $(C\underline{H}_2)_8CH_3$); 0.83 (3H, t, ^{3}J = 6.9, CH_3)
3f	6.80 (1H, d, ^{3}J = 8.2, H-2); 6.70 (1H, d, ^{3}J = 8.2, H-1); 5.84 (1H, m, β-CH-allyl); 5.22 (1H, d, ^{3}J = 17.3, γ-CH-allyl, *trans*); 5.12 (1H, d, ^{3}J = 10.7, γ-CH-allyl, *cis*); 5.04 (1H, s, 14-OH); 4.86 (1H, s, H-5); 3.11 (2H, m, NCH_2); 3.00 (1H, d, ^{3}J = 15.3, H-10*a*); 2.93 (1H, m, H-9); 2.86 (1H, m, H-7*a*); 2.56 (4H, m, H-10*e* + H-16*e* + $COCH_2$); 2.32 (1H, m, 15-H*a*); 2.06 (1H, m, H-7*e*); 1.92 (1H, m, H-16*a*); 1.74 (1H, m, H-8*e*); 1.59 (2H, m, $COCH_2C\underline{H}_2$); 1.40 (1H, m, H-8*a*); 1.22 (19H, m, H-15*e* + $(C\underline{H}_2)_9CH_3$); 0.82 (3H, t, ^{3}J = 6.5, CH_3)
3g	6.80 (1H, d, ^{3}J = 8.1, H-2); 6.70 (1H, d, ^{3}J = 8.1, H-1); 5.84 (1H, m, β-CH-allyl); 5.21 (1H, d, ^{3}J = 17.3, γ-CH-allyl, *trans*); 5.13 (1H, d, ^{3}J = 10.6, γ-CH-allyl, *cis*); 5.05 (1H, s, 14-OH); 4.87 (1H, s, H-5); 3.11 (2H, m, NCH_2); 3.00 (1H, d, ^{3}J = 15.1, H-10*a*); 2.93 (1H, m, H-9); 2.87 (1H, m, H-7*a*); 2.56 (4H, m, H-10*e* + H-16*e* + $COCH_2$); 2.31 (1H, m, 15-H*a*); 2.05 (1H, m, H-7*e*); 1.92 (1H, m, H-16*a*); 1.73 (1H, m, H-8*e*); 1.59 (2H, m, $COCH_2C\underline{H}_2$); 1.40 (1H, m, H-8*a*); 1.21 (21H, m, H-15 + $(C\underline{H}_2)_{10}CH_3$); 0.82 (3H, t, ^{3}J = 6.7, CH_3)
3h	6.80 (1H, d, ^{3}J = 8.2, H-2); 6.70 (1H, d, ^{3}J = 8.2, H-1); 5.85 (1H, m, β-CH-allyl); 5.22 (1H, d, ^{3}J = 17.7, γ-CH-allyl, *trans*); 5.13 (1H, d, ^{3}J = 10.4, γ-CH-allyl, *cis*); 5.03 (1H, s, 14-OH); 4.85 (1H, s, H-5); 3.13 (2H, m, NCH_2); 3.01 (1H, d, ^{3}J = 15.2, H-10*a*); 2.94 (1H, m, H-9); 2.87 (1H, m, H-7*a*); 2.56 (4H, m, H-10*e* + H-16*e* + $COCH_2$); 2.33 (1H, m, 15-H*a*); 2.06 (1H, m, H-7*e*); 1.94 (1H, m, H-16*a*); 1.75 (1H, m, H-8*e*); 1.60 (2H, m, $COCH_2C\underline{H}_2$); 1.41 (1H, m, H-8*a*); 1.22 (23H, m, H-15*e* + $(C\underline{H}_2)_{11}CH_3$); 0.83 (3H, t, ^{3}J = 6.7, CH_3)
3i	6.80 (1H, d, ^{3}J = 8.2, H-2); 6.70 (1H, d, ^{3}J = 8.2, H-1); 5.84 (1H, m, β-CH-allyl); 5.22 (1H, d, ^{3}J = 17.4, γ-CH-allyl, *trans*); 5.13 (1H, d, ^{3}J = 10.6, γ-CH-allyl, *cis*); 5.05 (1H, s, 14-OH); 4.86 (1H, s, H-5); 3.12 (2H, m, NCH_2); 3.00 (1H, d, ^{3}J = 15.1, H-10*a*); 2.94 (1H, m, H-9); 2.87 (1H, m, H-7*a*); 2.56 (4H, m, H-10*e* + H-16*e* + $COCH_2$); 2.32 (1H, m, H-15*a*); 2.06 (1H, m, H-7*e*); 1.93 (1H, m, H-16*a*); 1.74 (1H, m, H-8*e*); 1.59 (2H, m, $COCH_2C\underline{H}_2$); 1.41 (1H, m, H-8*a*); 1.21 (25H, m, H-15*e* + $(C\underline{H}_2)_{12}CH_3$); 0.82 (3H, t, ^{3}J = 6.8, CH_3)

tridecanoic acid **3f**. After a single injection this compound was able to block the narcotic effect of morphine for 19 days. An increase or, on the other hand, a decrease in the length of the hydrocarbon chain in the acid part of the 3-O-acyl naloxone derivative **3a-i** uniformly led to a stepwise fall in activity. It should be stressed that the behavioral signs of opioid intoxication with degradation of the effect of opiate receptor blockers arises earlier than changes in pain sensitivity characteristics and were thus the main factors for deciding the question of prolonging or ceasing the chronic experiment. In other words the differences between the experimental and control groups disappeared first in the behavioral signs of opioid intoxication and only then in indicators of pain sensitivity.

Thus the investigation carried out by us shows real promise in the creation of opiate receptor blockers with prolonged activity through the prodrugs obtained by acylation of naloxone with higher carboxylic acids.

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra for the naloxone base hydrate **2**, ^{1}H COSY NMR 2D spectroscopic experiments, homonuclear Overhauser NOESY-2D, and also heteronuclear HMQC and HMBC experiments were recorded on a Varian Mercury-400 spectrometer (400 and 100 MHz respectively). All 2D experiments were carried out with gradient selection of useful signals. Mixing times in the pulse sequences were $^{1}J_{CH}$ = 140 and $^{2\text{-}3}J_{CH}$ = 8 Hz. The number of increments in the COSY and HMQC spectra was 128 and 400 in the HMBC spectra. The mixing time in the NOESY-2D experiment was 500 ms. ^{1}H NMR spectra for the remaining compounds were recorded on a Bruker AC-300 instrument (300 MHz). In all cases TMS was used as internal standard. The content of the basic substance in the 3-O-acyl naloxone derivatives **3a-i** was determined by HPLC on a Waters Alliance 2690 liquid chromatograph with a Waters PAD 996 diode array detector. The chromatographic conditions were: Symmetry C8 (Nova Pak C8) column, size 3.9×150 mm; mobile phase flow rate 0.5 ml/min, column temperature 40ºC; injection volume 20 microliters; detection wavelength 230 nm.

Preparation of the mobile phase: Perchloric acid (6 ml), a 25% ammonia solution (5 ml), and 2-propanol (440 ml) were added to a solution of sodium heptylsulfonate (0.2 g) in water (100 ml) within a 1000 ml volumetric flask. The volume of the solution was then made to the mark with water and stirred. The solution was filtered under vacuum on a POR 16 (or similar) filter.

Naloxone hydrochloride dihydrate and ethyl oleate used in this work followed the requirements of the European pharmacopoeia [19].

Naloxone Base Hydrate 2. A 15% aqueous solution of sodium carbonate (10 ml) was added with stirring to a solution of naloxone hydrochloride dihydrate **1** (3.99 g, 0.01 mol) in water (20 ml). After several hours the precipitate was filtered off, washed with cold water, and dried in air. Recrystallization from acetone gave the naloxone base hydrate **2** with mp 173-175ºC. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm (*J*, Hz): 9.17 (1H, s, 3-OH); 6.55 (1H, d, ^{3}J = 8.0, H-2); 6.51 (1H, d, ^{3}J = 8.0, H-1); 5.82, 1H, m, β-CH allyl); 5.21 (1H, d, ^{3}J =16.8, γ-CH-allyl, *trans*); 5.12 (1H, d, ^{3}J = 10.0, γ-CH-allyl, *cis*); 4.95 (1H, s, 14-OH); 4.75 (1H, s, H-5); 3.10 (2H, m, NCH_2); 2.99 (1H, d, ^{3}J = 18.4, H-10*a*); 2.92 (1H, d, ^{3}J = 6.0, H-9); 2.88 (1H, m, H-7*a*); 2.48 (2H, d, H-10*e* + H-16*e*); 2.32 (1H, m, 15-H*a*); 2.06 (1H, m, H-7*e*); 1.95 (1H, m, H-16*a*); 1.71 (1H, m, H-8*e*); 1.43 (1H, m, H-8*a*); 1.27 (1H, m, H-15*e*). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 6.734 (1H, d, ^{3}J = 8.0, H-1); 6.606 (1H, d, ^{3}J = 8.0, H-2); 5.830 (1H, d, β-CH allyl); 5.224 (1H, d, ^{3}J = 18.8, γ-CH allyl, trans); 5.187 (1H, d, ^{3}J = 10.4, γ-CH allyl, *cis*); 4.697 (1H, s, H-5); 3.162 (2H, m, NCH_2); 3.091 (1H, d, ^{3}J = 18.8, H-10a); 3.050 (1H, d, $^{2}J = {}^{3}J_{aa}$ = 14.8, $^{3}J_{ae}$ = 5.2, H-7*a*); 3.013 (1H, d, ^{3}J = 6.0, H-9); 2.602 (1H, dd, ^{2}J = 12.0, ^{3}J = 4.8, H-16*e*); 2.550 (1H, dd, ^{2}J =18.8, ^{3}J = 6.0, H-10e); 2.408 (1H, m, $^{2}J = {}^{3}J_{aa}$ = 12.4, $^{3}J_{ae}$ = 5.2, H-15*a*); 2.310 (1H, m, ^{2}J = 14.4, $^{3}J_{ea} = {}^{3}J_{ee}$ = 3.2, H-7*e*); 2.173 (1H, m, $^{2}J = {}^{3}J_{ae}$ = 12.0, $^{3}J_{ae}$ = 3.6, H-16*a*); 1.886 (1H, m, ^{2}J = 13.2, $^{3}J_{ee}$ = 3.2, $^{3}J_{ea}$ = 5.2, H-8*e*); 1.630 (1H, m, $^{2}J = {}^{3}J_{aa}$ = 14.4, $^{3}J_{ae}$ = 3.2, H-8*a*); 1.575 (1H, m, ^{2}J = 12.8, ^{3}J = 2.8, H-15*e*). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 209.41 (C-6); 144.10 (C-4); 140.07 (C-3); 136.70 (β-C allyl);

130.08 (C-12); 124.01 (C-11); 119.68 (C-1); 118.17 (γ-C allyl); 117.88 (C-2); 90.01 (C-5); 70.53 (C-14); 62.31 (C-9); 57.68 (α-C allyl); 50.87 (C-13); 43.74 (C-16); 36.48 (C-7); 31.82 (C-8); 30.68 (C-15); 23.01 (C-10).

X-ray Structural Investigation. Crystals of the naloxone base hydrate **2** are rhombic (acetone), at 20ºC: a = 7.254(1), b = 13.907(1), c = 16.635(1) Å, V = 1678.0(1) Å^3, M_r = 345.38, Z = 4, space group $P2_12_12_1$, d_{calc} = 1.367 g/cm^3, μ(MoKα) = 0.099 mm^{-1}, F(000) = 736. The unit cell parameters and intensities of 7662 reflections (3707 independent with R_{int} = 0.020) were measured on an Xcalibur-3 diffractometer (MoKα radiation, CCD detector, graphite monochromator, ω-scanning to $2\theta_{max}$ = 55º).

The structure was solved by a direct method using the SHELXTL program package [20]. The positions of the hydrogen atoms were revealed from electron density difference synthesis and refined isotropically. The structure was refined by F^2 full matrix least squares analysis in the anisotropic approximation for non-hydrogen atoms to wR_2 = 0.078 for 3663 reflections (R_1 = 0.032 for 2909 reflections with $F > 4\sigma(F)$, S = 0.992). The full crystallographic data has been deposited in the Cambridge structural database (reference CCDC 672208). Interatomic distances and valence angles are given in Tables 1 and 2 respectively.

3-O-Acyl Derivatives of Naloxone (3a-i) (General Method). Methylene chloride (30 ml) was added to a solution of naloxone hydrochloride dihydrate **1** (3.99 g, 0.01 mol) in water (20 ml). Aqueous sodium carbonate (20%, 20 ml) was then added and thoroughly stirred. Stirring was continued and without allowing too vigorous evolution of CO_2 a solution of the corresponding carboxylic acid chloride (0.011 mol) in methylene chloride (10 ml) was added (external cooling was needed for large quantities). After addition of all of the acid chloride the reaction mixture was stirred for 1.5-2 h at room temperature. The organic layer was separated and the solvent removed (finally *in vacuo*). The residue was dissolved with heating in the appropriate solvent (see Table 3) and purified using carbon. The solution obtained was allowed to stand for several hours at room temperature. After that time the bulk of the substance crystallized (sometimes addition of a seed was needed to initiate the process) and held for 2-3 h at +5ºC. The crystals of the 3-O-acyl naloxone derivative **3** were filtered off, washed with hexane, and dried in a vacuum desiccator at room temperature.

Determination of the Opioprotective Activity of Compounds 3a-i was carried out in nonpedigree white rats. Two groups of experimental animals were used for evaluation of the length of the blocking action. The test ester **3a-**i (250 mg) was introduced subcutaneously as 1 ml of a sterile solution in ethyl oleate into each animal of the test group (24 in the studied sample). Animals in the control group received 1 ml of ethyl oleate as placebo. After 3 days all of the animals in the control group and three randomly chosen from the test group were administered subcutaneously a 1% solution of morphine at a dose of 30 mg/kg. After each addition of morphine animal observation was carried out over 2 h. In this way seven timed (5, 10, 20, 40, 60, 90, and 120 minutes) observations of the severity of the opioid intoxication [21, 22] for rodents were assessed for the behavioral signs of opioid intoxication as well as the state of their pain sensitivity according to Hafner (*via* mechanical constriction of the proximal part of the tail) [23].

For comparative characteristics of the action of the 3-O-acyl naloxones **3a-i** the results displayed after 2 h of the seven observations of the animals as ratings were summed each time and for three experimental animals simultaneously participating in experiment. The period between the day of injection and the day when the difference in reaction to morphine in the animals taken from the test and control groups stopped being significant was taken as the time of action of the investigated samples (the significance of the difference was calculated following the criteria of Wilcoxon, Mann, and Whitney [24]).

The absence of an opioid protective action in ethyl oleate was observed even with the first introduction of morphine in the control group. Hence in subsequent testing the opioid dose was introduced at an interval of 2-3 days only with test group animals randomly chosen in threes. To avoid the formation of tolerance to morphine each animal was administered the opioid once and was sacrificed using ether after recording the behavioral reactions and observation of pain sensitivity.

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