Narcotic Antagonists. 1. Isomeric Sulfate and Acetate Esters of Naloxone (*N*-Allylnoroxymorphone)

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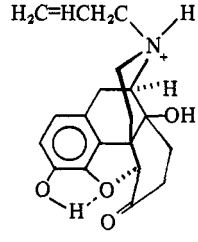
The synthesis of the two isomeric monosulfates **1b,c** and the disulfate **1d** esters of naloxone is described. These and the corresponding acetates **1e-g** were prepared as potentially longer acting narcotic antagonists than naloxone itself. The sulfation and acylation reactions appeared to reflect group interactions within the naloxone molecule. Intravenous administration to rats showed the acetates to have the same range of antagonistic potency as naloxone, with respect to the reversal of morphine-induced respiratory depression. By oral administration the acetates appeared to be several-fold more potent as well as longer acting than naloxone in preventing respiratory depression induced by morphine. In both intravenous and oral administration to rats, the sulfate esters proved inferior to naloxone in both potency and duration of action. Neither the acetates nor sulfates had any agonistic properties at relatively high iv dosages.

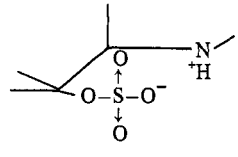
A promising form of chemical therapy for narcotic addiction involves the use of narcotic antagonists.[1] These agents block the sequelae of narcotic administration and serve to prevent readdiction following withdrawal. Many, if not all, of the existing narcotic antagonists are, however, of limited value since they retain some agonist activity and therefore possess a measure of addictive and psychotomimetic potential.[2-4] An exception is the potent and pure antagonist naloxone (*N*-allylnoroxymorphone)[5,6] which is free of agonist activity and is also devoid of significant side effects in man.[7,8] Naloxone, however, suffers from the disadvantages of a short duration of action when injected[3] and of a low potency when given orally.[5,9] Therefore, extending the effective life and increasing the oral effectiveness, while preserving the specific nature of naloxone activity, would represent a vast improvement for this form of postaddict chemical therapy. Our initial attempt at achieving these aims envisioned the synthesis of naloxone derivatives which would be resistant to rapid metabolic inactivation and would only slowly be transformed *in vivo* to naloxone providing longer duration of action. Since the specificity of antagonist activity is extremely sensitive to structural changes,[10] we have at this stage avoided major modifications of the naloxone structure in the hope of preserving the qualitative nature of its biological activity.

By analogy to the natural estrogens whose sulfate esters have a prominent role in oral estrogen therapy,[11] the sulfate esters of naloxone appeared to be promising derivatives. Such sulfate esters were of additional interest since they represent potential natural metabolites of naloxone[12] and their synthesis would aid in the elucidation of the metabolism of this narcotic antagonist. The corresponding acetates were also of interest both for their intrinsic possibilities and also to serve as a structure-activity guide to other organic acid esters of naloxone.

Chemistry. Naloxone **1a** contains a phenolic and an aliphatic hydroxyl group at C-3 and C-14, respectively, allowing for two isomeric monosulfates **1b** and **1c** and a disulfate 1d ester. A recently described sulfating procedure[13] which uses carbodiimide as the dehydrating agent has been reported to react selectively under mild conditions with aliphatic and not phenolic hydroxyls. More drastic conditions were required to effect phenolic sulfation. When the mild sulfating conditions were applied to naloxone, a monosulfate was obtained which surprisingly turned out to be predominantly the phenolic 3- and not the 14-monosulfate ester. This reversal of the expected reactivity of the phenolic and aliphatic hydroxyls toward sulfation can perhaps be rationalized on the basis of the structural features of naloxone. Under the acidic conditions of the reaction the charge on the protonated nitrogen (structure A) hinders



A

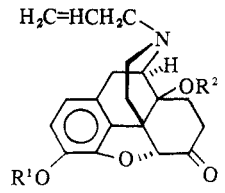


B

the approach of the electrophilic sulfur to the sterically proximal 14β-hydroxy group. Conversely, hydrogen bonding of the guiacol type (structure A) increases the nucleophilic character of the phenolic oxygen thus facilitating sulfation. When the more vigorous conditions, which were reported to yield phenolsulfates, were employed, the 3,14-disulfate of naloxone was obtained. The selective preparation of the 14-monosulfate isomer **1c** was best achieved by initially preparing the 3-monoacetate **1e**, as described below, which upon sulfation provided the 3-acetate 14-sulfate **1h**. Alkali hydrolysis of the latter removed the acetate but left the sulfate ester intact to provide the 14-monosulfate **1c** and thus complete the series of possible sulfate esters.

The 3-monoacetate **1e** of naloxone is readily prepared by the use of one equivalent of acetic anhydride in pyridine at room temperature. Little or no acetylation of the tertiary hydroxyl at C-14 takes place. Preparation of the 3,14-diacetate **1g** requires refluxing for 1 hr with an excess of acetic anhydride. Selective hydrolysis of the diacetate **1g** with 4% aqueous sulfuric acid for 20 hr at room temperature removes only the ester at C-3 and yields the 14-monoacetate **1f** in excellent yield. The stability of the 14-acetate to mild acid hydrolysis is a further reflection of its tertiary character and its spatial proximity to the protonated nitrogen. The 14-monoacetate **1f** is readily converted to the 3-sulfate 14-acetate **1i**, which upon alkali hydrolysis yields the 3-monosulfate, thus affording an alternate and more selective route to this compound.

The structures of the synthesized compounds were initially determined from the nmr spectra in deuterated dimethyl sulfoxide for the sulfate esters and in deuterated chloroform for the acetates. The data in Table I, in which the chemical shifts of the relevant protons are listed, reveal the consider-



1

a, $R^1 = R^2 = H$
b, $R^1 = SO_3^-$; $R_2 = H$
c, $R^1 = H$; $R_2 = SO_3^-$
d, $R^1 = R^2 = SO_3^-$
e, $R^1 = Ac$; $R^2 = H$
f, $R^1 = H$; $R^2 = Ac$
g, $R^1 = R^2 = Ac$
h, $R^1 = Ac$; $R^2 = SO_3^-$
i, $R^1 = SO_3^-$; $R^2 = OAc$

able effect of esterification of the 14β-hydroxyl group on the chemical shift of the trans 9α proton. This exceptionally large downfield shift may be the result of the cumulative effect of the chemical change of the 14-hydroxyl group and the resultant changes of its interaction with the piperidine nitrogen. The appearance of the shift is of important diagnostic value since it allows for ready identification of C-14 *vs.* C-3 substitution in the dihydroxy structure of naloxone.

The *N*-allyl group and the furan ring were shown to survive derivative formation by the identification of allyl resonance peaks (τ 4.8) and the C-5 proton resonance peak (τ 5.2) in both naloxone and its derivatives. The presence of the acetates and their positions was confirmed by an absorption at τ 7.77 and 7.90 for the 3- and 14-acetates, respectively. The ir spectra of the sulfate derivatives exhibited a broad band at 1250 cm^{-1} which was assigned to the asymmetrical S-O stretch of the sulfate group. The observation that each of the above derivatives under sufficiently drastic acid or base hydrolysis afforded naloxone provides final evidences that no skeletal changes occurred during their preparation.

The behavior of the isomeric monosulfates in dilute ammonium hydroxide (pH <10) is of some interest. Whereas the 3-monosulfate as well as 3,14-disulfate readily forms soluble salts in dilute ammonia solutions, the 14-monosulfate does not. Presumably an internal salt with the piperidine nitrogen (structure B) prevents salt formation with weak bases. The 14-sulfate does dissolve readily in higher pH solutions of NaOH either as the monosodium or as the disodium salt. The 3- and the 14-sulfates have also quite different rates of hydrolysis in acid, the 3-sulfate, as expected, being hydrolyzed far more rapidly.

Biological Activity. Preliminary biological data† on the above compounds were obtained from their reversal or prevention of morphine-induced respiratory depression in rats. Intravenous (iv) and oral activity was measured in anesthetized (sodium pentobarbital-urethane, ip) 200–300 g rats that were tracheotomized for mechanical ventilation, and the carotid artery was canulated for the measurement of respiratory rate and blood pressure. The jugular vein and gastric tract were canulated for iv and/or oral administration, respectively. Each study was carried out on three animals and the value reported is the arithmetical mean. The standard deviation was never more than 10% of the mean. The acetates were given as milligrams per kilogram of free base in 0.01 *N* HCl. The sulfates were given as ammonium or sodium salts.

Intravenous Activity. A dose of morphine sufficient to bring about respiratory arrest (12 mg/kg) was administered (iv) and the rat mechanically ventilated. Several minutes after respiratory arrest, the antagonist was given (iv) in small increments until a spontaneous respiratory rate returned. For each compound, the dose required to restore the respiration to about 80% of the control was determined. For naloxone it was found to be approximately 40 (±2) μg/kg, and the potency of each new compound was estimated relative to naloxone by taking the ratio of the naloxone dose to the dose of the test compound necessary to produce approximately the same degree of reversal of the depressed respiration. The results of some typical iv experiments are given in Table II. The mono- and disulfate, the 3-sulfate 14-acetate, and the diacetate esters were less potent than naloxone. The 3- and 14-monoacetates, however, were at least equipotent to naloxone.

Oral Activity. A 20 mg/kg dose of the antagonist was administered orally and subsequently challenged with a morphine dose (18 mg/kg, iv) at 60 and 240 min that would normally cause respiratory arrest. The degree to which a given compound prevents respiratory depression was taken as a measure of its antagonistic potential. Such experiments showed that neither naloxone nor any of its sulfate derivatives could prevent less than 50% respiratory arrest when challenged by morphine at either 60 or 240 min. The 3-acetate **1e** and the 14-acetate **1f** and the 3,14-diacetate **1g** maintained the respiratory rate at 100, 60, and 50% of control, respectively, after a morphine challenge at 60 min and continued to maintain the respiratory rate at 60, 0, and 0% of control, respectively, upon being challenged again at 240 min by an additional morphine dose. Thus, the acetates seem to have the same potency range as naloxone upon iv administration with respect to the reversal of morphine-in-

†Complete biological data will be published elsewhere when available.

Table I. H Chemical Shifts (τ) of the Sulfate and Acetate Esters of Naloxone

	H position			
	1	2	5	9
Naloxone[b]	3.42	3.42	5.20	6.7 (±0.5)[a]
3-Sulfate	3.20 (doublet)	2.65 (doublet)	5.00	6.7 (±0.5)[a]
14-Sulfate	3.42	3.42	5.05	5.45 (doublet)
3,14-Sulfate	3.20 (doublet)	2.60 (doublet)	5.00	5.45 (doublet)
3-Sulfate 14-acetate	3.20 (doublet)	2.60 (doublet)	5.50	5.40 (doublet)
	1,2 (AB quartet)			
Naloxone[c]	3,33, 3.45, 3.20, 3.10		5.30	7.0 (±0.5)[a]
3-Acetate	3.28, 3.38, 3.20, 3.10		5.38	7.0 (±0.5)[a]
14-Acetate	3.33, 3.45, 3.20, 3.10		5.35	5.65 (doublet)
3,14-Acetate	3.28, 3.38, 3.20, 3.10		5.35	5.65 (doublet)

[a]Superimposed on other methylene proton absorption bands and therefore exact resonance is not assignable. [b]DMSO-d_6. [c]$CDCl_3$.

Table II. Biological Activity of the Sulfate and Acetate Esters of Naloxone

Compound	Iv potency [a,b]
Naloxone (**1a**)	1.0
3-Acetate (**1e**)	2.0
14-Acetate (**1f**)	1.5
3,14-Diacetate (**1g**)	0.66
3-Sulfate (**1b**)	0.22
14-Sulfate (**1c**)	0.10
3,14-Disulfate (**1d**)	0.09
3-Sulfate 14-acetate (**1i**)	0.25

[a]Naloxone dose divided by the dose of the test compound needed to produce approximately the same degree of reversal of morphine (iv) depressed respiration. [b]Each value is an arithmetical mean of three separate determinations with a standard deviation never greater than 8% of the mean.

duced respiratory depression, but by the oral route these same acetates appeared to be more potent and may have a longer duration of action than orally administered naloxone.

None of the derivatives tested had any agonistic effect on the respiratory rate at five times the intravenous antagonist dose. These data were generated in the same three rats used to determine antagonistic potency subsequent to the completion of that part of the experiment. Though the biological data should only be considered as tentative, it is felt that they indicate the antagonistic potential, as well as the duration of action of various classes of naloxone derivatives.

Experimental Section

All melting points were taken on a Fisher-Johns apparatus and are uncorrected. Ir spectra were obtained on a Beckman IR-9. Nmr spectra were recorded on Varian Associates Model A-60 spectrophotometer; TMS was used as the internal standard. Analyses were determined by Spang Microanalytic Laboratory, Ann Arbor, Mich. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within 0.4% of the theoretical value.

Naloxone hydrochloride was obtained from Endo Laboratories. The hydrochloride was used as such in the sulfation procedures but for the synthesis of the acetate derivatives naloxone free base was required. The free base was made by precipitating naloxone from the aqueous solution of its HCl salt by the addition of 10% NH_4OH. The free naloxone was washed with distilled water and dried *in vacuo* at 58° for 24 hr. Dicyclohexylcarbodiimide was purchased from Aldrich Co. and used without further purification.

Naloxone 3-Acetate (1e). A mixture of naloxone, 300 mg (0.98 mmol), and acetic anhydride (0.11 ml, 1.18 mmol) in pyridine (3.0 ml) was allowed to stand for 24 hr at room temperature. H_2O (20 ml) was then added and the solution basified (pH 8) with 10% NH_4OH and rapidly extracted with $CHCl_3$. The $CHCl_3$ layer was washed twice with 5% NaOH and then with water. The organic layer was dried (Na_2SO_4) and evaporated under reduced pressure to give a white powder that resisted crystallization. Trituration with ether gave 264 mg of semicrystalline naloxone 3-acetate: mp 56–57°; tlc (silica gel G) 45:5:1 EtOAc–EtOH–AcOH, R_f 0.57; ir (KBr) 1760 cm^{-1} (3-acetate, C=O); nmr ($CDCl_3$) τ 3.28, 3.38, 3.20, 3.10 (1 H, 2 H AB multiplet), 5.38 (5 H), and 7.27 (3-acetate methyl H). *Anal.* ($C_{21}H_{23}NO_5 \cdot H_2O$) C, H, N.

Naloxone 3,14-Diacetate (1g). A solution of naloxone, 200 mg (0.918 mmol), in acetic anhydride (8 ml) was refluxed for 1 hr. The solution was evaporated under reduced pressure and the residue redissolved in $CHCl_3$ (20 ml) and washed several times with 5% NaOH and then water. The organic layer was dried (Na_2SO_4) and then evaporated under reduced pressure. The residue was crystallized twice from methanol to give 251 mg of naloxone 3,14-diacetate as plates: mp 149–159°; tlc (silica gel G) 45:5:1 EtOAc–EtOH–AcOH, R_f 0.93; ir (KBr) 1745 (14-acetate, C=O) and 1760 cm^{-1} (3-acetate, C=O); nmr ($CDCl_3$) τ 3.28, 3.38, 3.20, 3.10 (1 H, 2 H AB multiplet), 5.35 (5 H), 5.65 (9 H doublet), 7.77 (3-acetate methyl H), and 7.90 (14-acetate methyl H). *Anal.* ($C_{23}H_{25}NO_6 \cdot H_2O$) C, H, N.

Naloxone 3,14-diacetate (**1g**, 10 mg) on standing in 2 ml of aqueous NH_4OH (pH 11) for 4 hr at room temperature yielded a product which crystallized from ethyl acetate and was identical in all respects with naloxone.

Naloxone 14-Acetate (1f). Naloxone 3,14-diacetate (**1g**), 100 mg (0.255 mmol), was dissolved in 10 ml of 4% aqueous H_2SO_4 and allowed to stand for 24 hr at room temperature. The solution was then basified (pH 8) with 10% NH_4OH and extracted with 40 ml of $CHCl_3$. The organic layer was dried (Na_2SO_4) and evaporated under reduced pressure. The residue was crystallized from methanol to give 52 mg of naloxone 14-acetate: mp 193–194°; tlc (silica gel G) 45:5:1 EtOAc–EtOH–AcOH, R_f 0.88; ir (KBr) 1745 cm^{-1} (14-acetate, C=O); nmr ($CDCl_3$) τ 3.33, 3.45, 3.20, 3 10 (1 H, 2 H AB multiplet), 5.35 (5 H), 5.65 (9 H doublet), and 7.90 (14-acetate methyl H). *Anal.* ($C_{21}H_{23}NO_5 \cdot H_2O$) C, H, N.

Naloxone 3-Sulfate 14-Acetate 3-Ammonium Salt (1i). Naloxone 14-acetate (**1f**), 100 mg (0.259 mmol), and dicyclohexylcarbodiimide (DCC) were dissolved in 2 ml of dimethylformamide (DMF) and cooled to 0°. A chilled 1-ml solution of H_2SO_4 (0.029 ml, 0.518 mmol) in DMF was then added and the mixture allowed to stand for 15 min at 0° with occasional stirring. The reaction mixture was then basified (pH 9) with dilute NH_4OH and filtered. The filtrate was evaporated under reduced pressure and the residue redissolved in DMF (2 ml) and filtered to remove inorganic salts. EtOAc (10 ml) was added to the filtrate to give 88 mg of white semicrystalline naloxone 3-sulfate 14-acetate 3-ammonium salt: mp ~250° dec; tlc (silica gel G) 5:5:1 EtOAc–EtOH–NH_4OH, R_f 0.52; ir (KBr) 1250 (OSO_3), 1740 cm^{-1} (14-acetate, C=O); nmr (DMSO-d_6) τ 3.20 (1 H, doublet), 2.60 (2 H, doublet), 5.05 (5 H), and 5.40 (9 H, doublet). *Anal.* ($C_{21}H_{26}N_2O_8S \cdot H_2O$) C, H, N, S.

Naloxone 3-Sulfate Ammonium Salt (1b). (a) By Direct Sulfation of Naloxone. Naloxone hydrochloride, 200 mg (0.612 mmol), and DCC, 600 mg (3.0 mmol), were dissolved in 13.0 ml of DMF and cooled to 0°. A chilled solution of H_2SO_4 (0.031 ml, 0.55 mmol) in 1 ml of DMF was then added and the mixture allowed to stand for 15 min at 0° with occasional stirring. The reaction mixture is basified (pH 10) with 10% NH_4OH and filtered. The filtrate was evaporated under reduced pressure and the residue dissolved in DMF (2 ml) and filtered to remove inorganic salts. EtOAc (10 ml) was added to the filtrate to give a white semicrystalline precipitate. The precipitate was washed with $CHCl_3$ and a small quantity of EtOH to give 170 mg of naloxone 3-sulfate ammonium salt: mp ~250° dec; tlc (silica gel G) 5:5:1 EtOAc–EtOH–NH_4OH, R_f 0.42; ir (KBr) 1250 cm^{-1} (OSO_3); nmr (DMSO-d_6) τ 3.20 (1 H, doublet), 2.60 (2 H, doublet), 5.00 (5 H). *Anal.* ($C_{19}H_{24}N_2O_7S \cdot H_2O$) C, H, N, S.

(b) By Basic Hydrolysis of Naloxone 3-Sulfate 14-Acetate (1i). Naloxone 3-sulfate 14-acetate ammonium salt (**1i**, 20 mg) was added to 10 ml of dilute NH_4OH (pH 9), allowed to stand for 2 hr at room temperature, and then evaporated under reduced pressure. The residue was dissolved in DMF (2 ml), and EtOAc was then added to precipitate 15 mg of material that was identical in all respects with naloxone 3-sulfate ammonium salt as obtained by procedure a.

Naloxone 14-Sulfate (1c). Naloxone 3-acetate (**1e**), 54 mg (0.143 mmol), was dissolved in 2 ml of DMF and cooled to 0°. A chilled 1-ml solution of H_2SO_4 (0.043 ml, 0.775 mmol) in DMF was then added and the mixture allowed to stand for 15 min at 0° with occasional stirring. The reaction mixture was basified (pH 9) with dilute NH_4OH, filtered, and allowed to stand for 3 hr at room temperature to achieve hydrolysis of the 3-acetate ester. The filtrate was then evaporated under reduced pressure and the residue dissolved in DMF (2 ml) to remove inorganic salts. Ether (20 ml) was added to the filtrate to give a white precipitate which crystallized from H_2O to give 26 mg of naloxone 14-sulfate: mp >300°; tlc (silica gel G) 5:5:1 EtOAc–EtOH–NH_4OH, R_f 0.31; ir (KBr) 1250 cm^{-1} (OSO_3); nmr (DMSO-d_6) τ 3.42 (1 H and 2 H), 5.05 (5 H), and 5.45 (9 H, doublet). *Anal.* ($C_{19}H_{21}NO_7S \cdot H_2O$) C, H, N, S.

Naloxone 3,14-Disulfate 3-Ammonium Salt (1d). Naloxone hydrochloride, 200 mg (0.612 mmol), and DCC, 1.13 g (5.5 mmol), were dissolved in DMF (3.0 ml) and cooled to 0°. A chilled 1-ml solution of H_2SO_4 (0.183 ml, 3.3 mmol) in DMF was then added and the mixture allowed to stand 15 min at 0° with occasional stirring. The reaction was basified (pH 9) with 10% NH_4OH and filtered. The filtrate was evaporated under reduced pressure, and the residue was then dissolved in DMF (2 ml) and filtered to remove inorganic salts. EtOAc (10 ml) was added to the filtrate to give 208 mg of naloxone 3,14-disulfate 3-ammonium salt as a white semicrystalline powder: mp 143.5–145°; tlc (silica gel G) 5:5:1 EtOAc–EtOH–NH_4OH, R_f 0.18; ir (KBr) 1250 cm^{-1} (OSO_3); nmr (DMSO-d_6) τ 3.20 (1 H, doublet), 2.65 (2 H, doublet), 5.00 (5 H), and 5.45 (9 H, doublet). *Anal.* ($C_{19}H_{24}N_2O_{10}S_2 \cdot 2H_2O$) C, H, N, S.

Sequential Acid Hydrolysis of Naloxone 3,14-Disulfate 3-Ammonium Salt (1d). The sequential hydrolysis of naloxone disulfate **1d** was studied by dissolving 10 mg of compound **1d** in 4% D_2SO_4 (10 ml) and following the course of the reaction by nmr and tlc. After 24 hr at room temperature naloxone 14-sulfate was the main product, while after 48 hr complete hydrolysis to naloxone was effected.

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Notes

Narcotic Antagonists. 2. Preparation and Biological Stability of Naloxone-7,8-^3H

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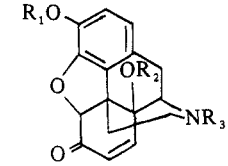
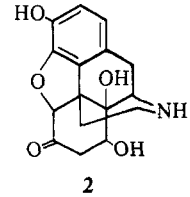
Naloxone (*N*-allylnoroxymorphone, **3b**) is a potent narcotic antagonist[1] which is in current clinical use as an antidote for narcotic overdosage† and is also a promising agent in the chemical treatment of narcotic addiction.[2] It is unique in that it is a pure antagonist devoid of any agonist activity,[3] and it is therefore of particular importance in the study of the biochemistry of narcotic action and addiction. To permit detailed studies of the metabolism and disposition of naloxone in experimental animals and in man,‡ a labeled substrate with the isotope located in biologically stable positions was required. A high specific activity of at least 10 Ci/mmol was desirable to allow for the use of the labeled material in contemplated protein binding studies.

Reduction of a double bond with tritium gas is a most effective method of achieving high specific activity labeling. Since the synthesis of naloxone from thebaine involves the reduction of an unsaturated intermediate,[5] this procedure was applicable in this instance subject to two principal considerations. One was that the tritium reduction had to be carried out prior to the introduction of the *N*-allyl group, and the other was that to ensure practical yields a minimum of chemical manipulation subsequent to the isotope incorporation be required. The above requirements were best realized by the reduction of 14-hydroxynormorphinone (**1d**) with tritium which then needed only N-allylation to convert it to naloxone. 14-Hydroxynormorphinone (**1d**) was obtained from 14-hydroxymorphinone (**1a**) by the following sequence.

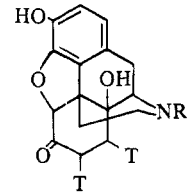
14-Hydroxymorphinone (**1a**)[6] was converted to its diacetate **1b** in refluxing acetic anhydride. Reaction of the diacetate **1b** with CNBr led to the *N*-cyano derivative **1c**. Removal of the cyano and acetate groups in **1c** by acid hydrolysis proved to be troublesome in that infrared analysis of the product revealed the presence of variable proportions of a saturated keto compound. By analogy to the 14-hydroxymorphinone case,[6] the contaminant was probably derived from hydration of the 7,8-double bond and was assigned the 8,14-dihydroxydihydronormorphinone (**2**) structure. The most satisfactory conditions for the hydrolysis of **1c** were found to be refluxing in 20% HCl for 3 hr. These conditions were sufficient to effect removal of both the cyano and acetate groups and yielded a product containing 14-hydroxynormorphinone (**1d**) and 8,14-dihydroxydihydronormorphinone (**2**) in 1:1 proportion. The mixture proved to be difficult to separate and was reduced with tritium in the presence of 10% palladized charcoal without separation. Since the 8,14-dihydroxy component **2** of the mixture contained no aliphatic unsaturation, it was unaffected by the reduction and only the 14-hydroxynormorphinone incorporated the isotope to give 14-hydroxydihydronormorphinone-7,8-^3H (**3a**), without major radiochemical impurities. The product of the tritium reduction was then allowed to react with allyl bromide to yield naloxone-7,8-^3H (**3b**) in about 40% yield. The latter when purified by preparative tlc or by gradient elution partition chromatography on Celite provided material with a specific activity of 40 Ci/mmol, which by reverse isotope dilution with inert carrier naloxone was better than 97% pure.



1a, R_1, R_2 = H; R_3 = CH_3
b, R_1, R_2 = Ac; R_3 = CH_3
c, R_1, R_2 = Ac; R_3 = CN
d, R_1, R_2 = R_3 = H



2



3a, R = H
b, R = $CH_2CH{=}CH_2$

The biological stability of the isotope in both the C-7 and C-8 positions of naloxone was confirmed by the following experiment. After the administration of 10×10^6 cpm of the above naloxone-^3H to a volunteer male subject, urine was collected for 3 days. Lyophilization of an aliquot of the combined urines yielded water with a specific activity of 1 cpm/ml. Since the total body water volume of the subject was 35 l., 35,000 cpm or only 0.35% of the dose

†"Narcan," Endo Laboratories, Inc., Garden City, N. Y.

‡The urinary metabolites of inert naloxone have been reported on in the chicken and rabbit[4a] and in man.[4b]