

1

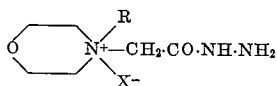
2

3,398,147
MORPHOLINOACETOHYDRAZIDE ALKYL HALIDES AND PROCESS FOR THEIR PREPARATION

Minoru Tohda, Noriaki Kuwana, and Kiyoshi Kawabe, Tokyo, Japan, assignors to Eisai Co., Ltd., Tokyo, Japan, a corporation of Japan
 No Drawing. Filed June 18, 1965, Ser. No. 465,173
 8 Claims. (Cl. 260—247.2)

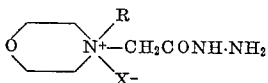
ABSTRACT OF THE DISCLOSURE

A morpholinoacetohydrazide alkyl halide of the formula:

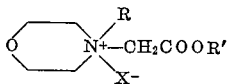


wherein R stands for an alkyl group having 1 to 4 carbon atoms and X stands for a halogen.

A process for producing a morpholinoacetohydrazide alkyl halide of the formula:



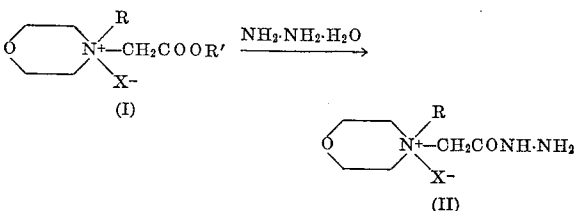
wherein R stands for an alkyl group having 1 to 4 carbon atoms and X stands for a halogen, comprising reacting an alkyl morpholinoacetate alkyl halide of the formula:



wherein R and R' stand for alkyl groups having 1 to 4 carbon atoms and X stands for a halogen, with hydrazine hydrate.

This invention relates to morpholinoacetohydrazide alkyl halides, new ketone reagents and processes for synthesizing them.

Morpholinoacetohydrazide alkyl halides which we have discovered have the following Formula II and can be made by reacting an alkyl morpholinoacetate alkyl halide of the following Formula I with hydrazine hydrate. This reaction is represented by the following equation:



wherein R and R' stand for alkyl groups having 1-4 carbon atoms, such as methyl, ethyl and butyl, and X stands for a halogen, such as chlorine, bromine and iodine.

The alkyl morpholinoacetate alkyl halide (I) which is a starting material for obtaining the new compounds of the present invention can be easily obtained by making an alkyl halogenoacetate react on an N-alkyl morpholine or making an alkyl halide react on alkyl morpholinoacetate. The morpholinoacetohydrazide alkyl halogenide of this invention can be easily obtained by stirring or shaking for a while the thus obtained alkyl morpholinoacetate alkyl

halide together with hydrazine hydrate in a little excess of the calculated amount in an organic solvent, such as methanol or ethanol. The reaction proceeds within a short time when carried out at room temperature.

The morpholinoacetohydrazide alkyl halides have not been known heretofore and they are useful as new ketone reagents. We have discovered that when they are added to a solution of a mixture of Δ^4 -3-keto-steroids and $\Delta^{1,4}$ -3-keto-steroids, they combine selectively with the Δ^4 -3-keto-steroids to yield water-soluble derivatives and are therefore very valuable as separating agents of $\Delta^{1,4}$ -3-keto-steroids.

It is well known that when double bonds are introduced into the C₁-C₂ positions of cortisone and hydrocortisone, the corticoidal activity of these compounds increases and that, when a double bond is introduced into the C₁-C₂ position of testosterone or methyltestosterone, the anabolic activity of these compounds increases. Thus, the introduction of a double bond into the C₁-C₂ position has become important to steroid compounds.

There are several methods of synthesizing $\Delta^{1,4}$ -3-keto-steroids from Δ^4 -3-keto-steroids. For example, they are made by dehydrogenating with selenium dioxide or with various microbes such as *Didymella lycopersici*. Among these methods, the microbiological dehydrogenation is one of the most useful methods, due to the facts that the reaction proceeds relatively specifically and that its conditions are mild and have a smaller loss of steroids. However, this method has a defect that, in most cases, the unreacted Δ^4 -3-keto-steroid will be mixed with the desired $\Delta^{1,4}$ -3-keto-steroid. The physical and chemical properties of the desired steroid are so similar to those of the starting steroid that, even if a separation is attempted by adopting such usual methods as chromatography or fractional crystallization in order to obtain the pure $\Delta^{1,4}$ -3-keto-steroid from such mixture, it will often be very difficult to carry out the separation effectively.

We have discovered that morpholinoacetohydrazide alkyl halides have a very high ability for separating such a steroid mixture. That is to say, the morpholinoacetohydrazide alkyl halide forms a water-soluble derivative with any of Δ^1 -, Δ^4 - and $\Delta^{4,6}$ -3-keto-steroids but forms no derivative with a $\Delta^{1,4}$ -3-keto-steroid unless it has another reactive carbonyl group other than the 3-keto group.

Further, if not only a steroid but also a compound having a keto group react with a morpholinoacetohydrazide alkyl halide, a water-soluble derivative will be formed. Therefore, a compound having a reactive carbonyl group and a nonreactive compound can be easily separated from a mixture of both of them. The term "reactive carbonyl" means a carbonyl group that will form a derivative with a morpholinoacetohydrazide alkyl halide under the ordinary conditions. For example, the 11-keto group of a steroid is nonreactive with morpholinoacetohydrazide alkyl halides and the 20-keto group of cortisone is reactive but, when the hydroxyl group in the C₂₁ position is converted to an acetate, it will be subjected to a steric hindrance and will become nonreactive. Therefore, when a morpholinoacetohydrazide alkyl halide is used, a $\Delta^{1,4}$ -3-keto-steroid will be easily separated from a mixture with it of a saturated-3-keto-steroid, or a Δ^1 -, Δ^4 - or $\Delta^{4,6}$ -3-keto-steroid.

In separating $\Delta^{1,4}$ -3-keto-steroids from a steroid mixture, a morpholinoacetohydrazide alkyl halide is made to react with a steroid mixture in a proper solvent such as for example, a mixed solvent of methanol and acetic acid, and

then a $\Delta^{1,4}$ -3-keto-steroid can be favorably separated by any suitable method. As the thus formed morpholinoacetohydrazide alkyl halide derivative is water-soluble, it can be easily extracted from the reaction mixture with water. If the solvent used in the preparation of the derivative is water-insoluble, if water is added after the completion of the reaction, the $\Delta^{1,4}$ -3-keto-steroid will remain in the water-insoluble solvent, the derivative will move into the water layer and therefore they can be easily separated. Further, if a water-soluble solvent is used in the preparation of a morpholinoacetohydrazide alkyl halide derivative, if water is added after the completion of the reaction, the $\Delta^{1,4}$ -3-keto-steroid can be extracted with a water-insoluble solvent.

Further, it is desirable to neutralize the reaction mixture of a steroid mixture and a morpholinoacetohydrazide alkyl halide with an aqueous solution of a weak alkali, such as sodium bicarbonate, and then extract the $\Delta^{1,4}$ -3-keto-steroid with a water-insoluble solvent, such as benzene, ethyl acetate or chloroform. The reaction temperature may be from room temperature to the boiling point of the solvent.

The following Examples 1-3 show examples of the synthesis of morpholinoacetohydrazide alkyl halides of this invention and Examples 4-9 show examples of their use in the separation of steroids. However, these examples are described to illustrate further the present invention but not to limit it.

EXAMPLE 1

Synthesis of morpholinoacetohydrazide methyl chloride.—To a solution of 21 g. (0.24 mol) of N-methyl morpholine in 50 ml. of absolute ethanol, were added 25 g. (0.22 mol) of ethyl monochloracetate and the mixture was heated under reflux for about 10 hours. Immediately after the reaction was completed, the ethanol was distilled away under a reduced pressure and then the residue was obtained as a solid. The pulverized residue was washed with benzene. When this residue was recrystallized from a mixed solution of ethanol and benzene, there was obtained 41 g. of ethyl morpholinoacetate methyl chloride (M.P., 168 to 169° C.) in the form of white granules. To a solution of 31 g. (0.14 mol) of the ester, thus obtained, in 100 ml. of absolute ethanol, were added 7.7 g. (0.154 mol) of 100% hydrazine hydrate. When the mixture was vigorously shaken, in a while, it became turbid to white and oily hydrazide was separated. After it was left overnight and then the vessel wall was rubbed, the oily hydrazide gradually crystallized. The isolated crystals were dipped in absolute ethanol, crushed in a mortar and then separated by filtration. When they were then recrystallized from dilute ethanol, there were obtained 23 g. (71.9%) of morpholinoacetohydrazide methyl chloride (M.P. 185-186.5° C. dec.) in the form of white prisms.

Analysis.—Calcd. for $C_7H_{16}O_2N_3Cl$: C, 40.07%; H, 7.69%; N, 20.03%; Cl, 16.88%. Found: C, 40.20%; H, 7.74%; N, 19.65%; Cl, 17.36%.

EXAMPLE 2

Synthesis of morpholinoacetohydrazide methyl bromide.—To a solution of 20 g. (0.198 mol) of N-methyl-morpholine in 50 ml. of absolute ethanol were added 33 g. (0.198 mol) of ethyl bromacetate. The mixture was heated under reflux on a water bath for 1.5 hours. After the reaction was completed, the mixture was cooled. 11 g. (0.22 mol) of 100% hydrazine hydrate were added directly to the reaction solution without isolating ethyl morpholinacetate methyl bromide thus obtained. When the mixture was vigorously shaken, the desired hydrazide was deposited at first as an oily product. By a method identical to that described in Example 1, there were obtained 44 g. (87.3%) of morpholinoacetohydrazide methyl bromide (M.P. 193-194° C., dec.) in the form of white needles.

Analysis.—Calcd. for $C_7H_{16}O_2N_3Br$: C, 33.12%; H, 6.35%; N, 16.54%; Br, 31.46%. Found: C, 33.41%; H, 6.0%; N, 16.32%; Br, 31.25%.

EXAMPLE 3

Synthesis of morpholinoacetohydrazide methyl iodide.—To a solution of 11 g. (0.11 mol) of N-methyl morpholine in 50 ml. of absolute ethanol were added 22.3 g. (0.104 mol) of ethyl iodacetate. In the same manner as in Examples 1 and 2, ethyl morpholinoacetate methyl iodide was obtained. When 6.5 g. (0.13 mol) of 100% hydrazine hydrate were added directly to the reaction solution and exactly the same operation as in the foregoing examples was carried out, there were obtained 26.3 g. (83.2%) of morpholinoacetohydrazide methyl iodide (M.P. 161 to 162° C.) in the form of white leaflets.

Analysis.—Calcd. for $C_7H_{16}O_2N_3I$: C, 27.93%; H, 5.36%; N, 13.96%; I, 42.17%. Found C, 28.22%; H, 5.62%; N, 14.08%; I, 41.69%.

EXAMPLE 4

Separation of a mixture of methyl testosterone and Δ^1 -methyl-testosterone with morpholinoacetohydrazide methochloride.—3.0 g. of methyl testosterone and 3.0 g. of Δ^1 -methyl testosterone were dissolved in 100 ml. of methanol-acetic acid (9:1). To the solution was added 2.70 g. of morpholinoacetohydrazine methochloride, whereupon the mixture was maintained at 40 to 43° C. for 1.5 hours with occasional shaking. After it was cooled, the reaction solution was slowly poured into 500 ml. (about 90% of the theoretical amount to neutralize) of an aqueous solution of 2.65% sodium bicarbonate and then saturated sodium bicarbonate aqueous solution was added to perfectly neutralize the solution. The solution was extracted twice with 300 ml. of benzene. The combined extracts were washed twice with 100 ml. of water, and dried over anhydrous sodium sulfate. After removal of the solvent under a reduced pressure, there was obtained 2.91 g. (97% of theoretical) of Δ^1 -methyl-testosterone having a melting point of 166 to 168° C. and λ max. of 244 μ (ethanol). 50 ml. of concentrated hydrochloric acid was added to the water layer left after the extraction with benzene and the acidic solution was left at the room temperature for two hours. Then the precipitated methyl testosterone was extracted twice with 300 ml. of benzene. The benzene extracts were combined, washed with a 1% aqueous solution of sodium bicarbonate, next with water, and dried over anhydrous sodium sulfate. After removal of the solvent under a reduced pressure, there was obtained 2.87 g. (95.7%) of methyl testosterone melting at 163 to 165° C. of λ max. of 241 μ (ethanol).

EXAMPLE 5

Separation of a mixture of methyl testosterone and Δ^1 -methyl testosterone with morpholinoacetohydrazide methobromide.—To a solution of 3.0 g. of methyl testosterone and 3.0 g. of Δ^1 -methyl testosterone in 100 ml. of methanol-acetic acid (9:1), was added 3.0 g. of morpholinoacetohydrazide methobromide. The mixture was held at 40 to 43° C. for 2.5 hours with occasionally shaking. Thereafter, by a method identical to that described in Example 4, there was obtained 2.82 g. (94% of theory) of Δ^1 -methyl testosterone which had a melting point of 167 to 169° C. and λ max. of 244 μ (ethanol) and 2.82 g. (yield: 94%) of methyl testosterone which had a melting point of 163 to 165° C. and λ max. of 241 μ (ethanol).

EXAMPLE 6

Separation of a mixture of methyl testosterone and Δ^1 -methyl testosterone with morpholinoacetohydrazide methiodide.—To a mixture of 3.0 g. of methyl testosterone and 3.0 g. of Δ^1 -methyl testosterone dissolved in 100 ml. of methanol-acetic acid (9:1), was added 3.6 g. of morpholinoacetohydrazide methiodide. The mixture was allowed to react at 40 to 43° C. for three hours and was shaken occasionally. Subsequently, in the same manner as in Example 1, there was obtained 2.81 g. (yield: 93.7%) of Δ^1 -methyl testosterone of a melting point of

5

166 to 168° C. and λ max. of 244 $m\mu$ (ethanol) and 2.83 g. (94.3% of theory) of methyl testosterone of a melting point of 163 to 165° C. and λ max. of 241 $m\mu$ (ethanol).

EXAMPLE 7

Separation of a mixture of testosterone and Δ^1 -testosterone with morpholinoacetohydrazide methochloride.—A solution was prepared of 3.0 g. of testosterone and 3.0 g. of Δ^1 -testosterone in 100 ml. of methanol-acetic acid (9:1). 2.7 g. of morpholinoacetohydrazide methochloride was added to the solution. The mixture was reacted at 40 to 43° C. for three hours while being shaken occasionally. When the separation by the operation as in Example 1 was then carried out, there were obtained 2.79 g. (93.0% of theory) of Δ^1 -testosterone of a melting point of 171 to 172° C. and λ max. of 244 $m\mu$ (ethanol) and 2.80 g. (93.3% of theory) of testosterone of a melting point of 155° C. and λ max. of 241 $m\mu$ (ethanol).

EXAMPLE 8

Separation of a mixture of hydrocortisone acetate and prednisolone acetate with morpholinoacetohydrazide methochloride.—The mixture consisting of 2.0 g. of hydrocortisone acetate and 2.0 g. of prednisolone acetate was boiled in 80 ml. of ethanol-acetic acid (9:1) until it dissolved. 1.2 g. of morpholinoacetohydrazide methochloride was added to the solution. The mixture was heated under reflux on a water bath for 30 minutes. After it was cooled, it was poured into 400 ml. of a 2.65% aqueous solution of sodium bicarbonate. Further, a saturated aqueous solution of sodium bicarbonate was added to the solution to perfectly neutralize it. Then the unreacted steroid was extracted twice with 400 ml. of ethyl acetate. The combined extract was washed with water, dried over anhydrous sodium sulfate and then was concentrated in vacuo yielding 1.87 g. (93.5% of theory) of colorless prisms. When a part of the crystals was recrystallized from ethanol, it showed a melting point of 235 to 237° C. and λ max. of 244 $m\mu$ (ethanol). Its infrared spectrum perfectly coincided with that of standard prednisolone acetate.

40 ml. of concentrated hydrochloric acid were added to the water layer left after the extraction with ethyl acetate. The mixture was allowed to stand for two hours, and extracted twice with 400 ml. of ethyl acetate. The combined extract was washed with an aqueous solution of sodium bicarbonate, and next with water and then dried over anhydrous sodium sulfate. After removal of the solvent in vacuo, 1.83 g. (91.5% of theory) of small colorless prisms were obtained. When a part of the crystals was recrystallized from ethanol, it showed a melting point of 222 to 223° C. and λ max. of 241 $m\mu$ (ethanol). Its infrared spectrum favourably coincided with that of standard hydrocortisone acetate.

EXAMPLE 9

Isolation of Δ^1 -methyl testosterone with morpholinoacetohydrazide methochloride from an extract of Δ^1 -dehydrogenation product of methyl testosterone by microbes.—A solution prepared by dissolving 30 g. of methyl testosterone in 200 ml. of methanol was aseptically added to the fermentation broth of *Diaporthe phaseolorum* precultured in 15 liters of Henneberg's culture medium (consisting of 10% sucrose, 0.2% KNO_3 , 0.1% KH_2PO_4 , 0.05% $MgSO_4 \cdot 7H_2O$ and 0.013% $CaCl_2 \cdot 2H_2O$) in a jar fermenter at 26.5° C. for two days and the culturing was further continued at 26.5° C. for four days. After the completion of the culturing, the culture solution was filtered. The collected mycelia were extracted with methanol and the extract was concentrated under a reduced pressure. The aqueous residue and the culture filtrate were combined and extracted with ethyl acetate. The extract was concentrated under a reduced pressure, giving 44 g. of a brown residue. A part of this residue was subjected to a paper chromatography so as to establish how the dehydrogenation had proceeded. After its ultraviolet absorbing parts were then cut off, extracted with ethanol and the optical

6

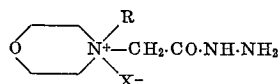
density of every extract was measured, it was found that about 14 g. of methyl testosterone and about 12 g. of Δ^1 -methyl testosterone were obtained.

This residue was dissolved in 220 ml. of methanol-acetic acid (9:1) and thereupon 20 g. of morpholinoacetohydrazide methochloride was added. The mixture was allowed to react at 40 to 43° C. for two hours while being shaken occasionally. After the reaction was completed, the reaction solution was neutralized by pouring it into 1.1 liters of 2.65% aqueous solution of sodium bicarbonate. The solution was then extracted twice with 1 liter of ethyl acetate. The combined extract was washed with water dried over anhydrous sodium sulfate and subsequently concentrated in vacuo giving an oily residue. The residue was dissolved in a small amount of acetone. When 12.3 g. of crude crystals precipitated after the solution was left standing overnight were recrystallized from acetone-ether, there were obtained 11.2 g. (37.2% of theory) of prisms of a melting point of 166 to 168° C. and λ max. of 244 $m\mu$ (ethanol). The infrared spectrum of this product perfectly coincided with that of the standard product Δ^1 -methyl testosterone.

100 ml. of concentrated hydrochloric acid were added to the water layer which remained after the solution was extracted with ethyl acetate. The mixture was left standing for two hours and was extracted twice with 1 liter of ethyl acetate. The combined extract was washed with an aqueous solution of sodium bicarbonate and with water, dried over anhydrous sodium sulfate and was then concentrated under a reduced pressure. The obtained concentrated residue was crystallized. When the crystals were recrystallized from acetone, there were obtained 13.6 g. of needles of a melting point of 163 to 165° C. and λ max. of 241 $m\mu$ (ethanol). The infrared spectrum of the recovered material was perfectly identical with that of the standard methyl testosterone.

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A morpholinoacetohydrazide alkyl halide of the formula:



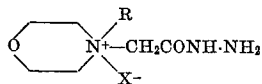
wherein R stands for an alkyl group having 1-4 carbon atoms and X stands for a halogen.

2. A morpholinoacetohydrazide alkyl halide as claimed in claim 1, wherein R is a methyl group.

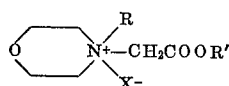
3. A morpholinoacetohydrazide alkyl halide as claimed in claim 1, wherein X is selected from the group consisting of chlorine, bromine and iodine.

4. A morpholinoacetohydrazide methyl halide as claimed in claim 2 wherein X is selected from the group consisting of chlorine, bromine and iodine.

5. A process for producing a morpholinoacetohydrazide alkyl halide of the formula:



wherein R stands for an alkyl group having 1 to 4 carbon atoms and X stands for a halogen, comprising reacting an alkyl morpholinoacetate alkyl halide of the formula:



wherein R and R' stand for alkyl groups having 1 to 4 carbon atoms and X stands for a halogen, with hydrazine hydrate.

6. A process as claimed in claim 5, wherein R is a methyl group.

7. A process as claimed in claim 5, wherein X is selected from the group consisting of chlorine, bromine and iodine.

8. A process as claimed in claim 6, wherein X is selected from the group consisting of chlorine, bromine and iodine.

References Cited

UNITED STATES PATENTS

1,915,334 6/1933 Salzberg et al. ----- 260—243
2,075,359 3/1937 Salzberg et al. ----- 167—22

OTHER REFERENCES

Toda et al., Chemical Abstracts, p. 1703a, vol. 62 (1965).

5 NICHOLAS S. RIZZO, *Primary Examiner*.
JOSE TOVAR, *Assistant Examiner*.