Tetrahedron Vol 27, pp. 1909 to 1915. Pergamon Press 1971 Printed in Great Britain

STEROIDAL ANALOGUES OF UNNATURAL CONFIGURATION—II[1]

AUTOXIDATION OF NEUTRAL $\Delta^{17(20)}$-ENOLS; A METHOD OF 17-HYDROXYLATION

P. R. ENSLIN

National Chemical Research Laboratory, C.S.I.R., Pretoria, Republic of South Africa

(*Received in the UK* 9 *November* 1970; *Accepted for publication* 19 *November* 1970)

Abstract—Hydrogenation of Δ^{16}-20-ketones, derived from cucurbitacins, with a neutral supported Pd catalyst gave relatively stable $\Delta^{17(20)}$-enols which readily autoxidised in benzene solution to 17-hydroperoxy-20-ketones. These intermediates could be reduced to 17-hydroxy-20-ketones by triethyl phosphite or converted to 17-ketones on heating. In model studies, 3β-acetoxy-17α-hydroxy-16β-methyl-pregn-5-en-20-one and 5α- and 5β-hydroxy-cholestan-6-ones were prepared by this method. The reactivity of αβ-unsaturated ketones is determined by a preferred 1,4-addition of hydrogen to give enols stabilized by electronic or steric factors.

THE catalytic hydrogenation of 3α-hydroxy-4,4,14α-trimethyl-19-nor-10α-pregn-5,16-diene-11,20-dione (I)[2] to its 16,17-dihydro derivative (II), was studied in detail after it was observed that the 17-ketone (III) was formed as a by-product. Hydrogenations were carried out in 96% ethanol with a 5% palladium on barium sulphate catalyst.

Initially the amount of 17-ketone formed was estimated from a 1740 cm^{-1} band in the IR spectra of the crude product. The results were, however, not reproducible which suggested that factors operating during the isolation of the crude product, were important. When the fate of the hydrogenation products were followed by GLC, a clear sequence of events emerged. Under suitable conditions (Experimental), the 17-ketone (III) and 20-ketone (II) were separated with retention times of 4 and 7 min, respectively.

The hydrogenation of the Δ^{16}-20-ketone (I) was complete after the absorption of 1 mole of hydrogen. No GLC evidence of an "oxidation product" was observed as long as the mixture was kept under hydrogen. After removal of the catalyst and exposure to air, a peak gradually developed at 4 min retention time, reaching a maximum (ca. 80% of total) after 24 hr. The addition of a trace of base to a freshly hydrogenated product completely inhibited the formation of this autoxidation product and gave only the 16,17-dihydro derivative (II).

Clearly the first product of the hydrogenation had to be the enol (IV) formed by a 1,4-addition of hydrogen to the enone system. This enol is capable of existing in the absence of the catalyst in a strictly neutral ethanolic solution. In air it was slowly attacked by oxygen to give the hydroperoxide (V) which decomposed thermally on the GLC column to give the 17-ketone (cf base catalysed autoxidation of steroidal 20-ketones to the corresponding 17-hydroperoxy-20-ketones[3] and their subsequent decomposition to 17-ketones[4]). In direct analogy Attenburrow *et al.*[5] reported the

isolation of a neutral crystalline $\Delta^{17(20)}$-enol in 28% yield on hydrogenation of a dilute solution of 3β-acetoxy-16-methyl-5α-pregna-9,16-dien-20-one in a mixture of tetrahydrofuran and triethylamine. This enol was readily autoxidised to the corresponding 17-hydroperoxy-20-ketone.

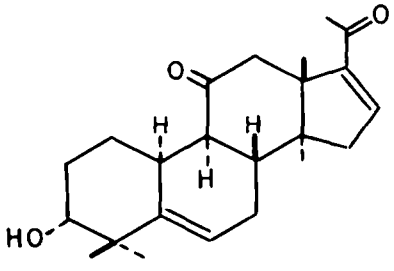
On addition of an excess of triethyl phospite[6] to an autoxidised solution of the hydrogenation product of the enone, the GLC peak at 4 min was replaced by a new peak at 9·3 min of the corresponding 17-hydroxy compounds, thus providing conclusive proof that a hydroperoxy-20-ketone must be the initial autoxidation product.

Various methods were studied to accelerate the autoxidation of the enol. The addition of trace amounts of hydrochloric or acetic acids, or heating the ethanolic solution to 70°, led to an initial increase in the rate of autoxidation which was, however, rapidly terminated by promotion of the competing ketonisation reaction to give the 16,17-dihydro-20-ketone (II). No increase in the rate of oxidation was found by bubbling oxygen through the solution. A very rapid conversion to the hydroperoxide was, however, readily achieved by evaporating the ethanol at room temperature under reduced pressure and dissolving the product in benzene or toluene. The reaction was complete within 5 min and the GLC peak at 4 min showed an 80% conversion. The thermal decomposition of the hydroperoxide (V) to the 17-ketone (III) was conveniently carried out by heating a toluene solution under reflux. This provided a useful preparative method for the 17-ketone.

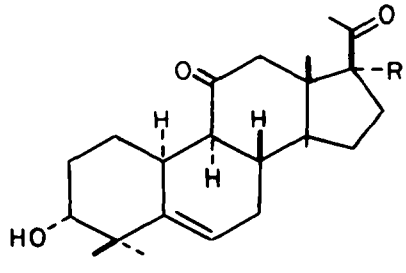
The addition of an excess of triethyl phospite to an autoxidised solution gave a 90% yield (GLC) of a mixture of the isomeric 17-hydroxy-20-ketones in a ratio of *ca* 5:1. The major isomer was isolated and is probably the 17α-hydroxy isomer (VI).

In experiments using a 5% palladium on calcium carbonate catalyst, the yield of oxidation product varied between 10 and 50% with different batches of catalyst. Extensive washing of the catalyst to remove traces of acid or base failed to improve the yield and it is considered that the physical state of the support has an influence on the ketonisation of the reactive enolic species.

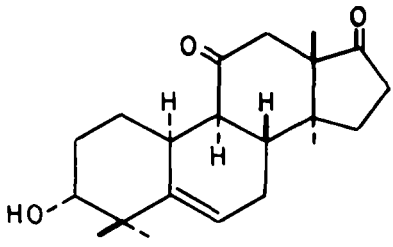
The preparative utility of this mild method of 17-hydroxylation was also investigated with a Δ^{16}-20-ketone derived from cucurbitacin B. Rings B and C are *cis*-fused (9β-methyl) in 3α-hydroxy-4,4,14α-trimethyl-19(10→9β)-*abeo*-pregna-5,16-diene-2,-11,20-trione (VII).[1] The presence of a base-sensitive α-ketol system also provided a



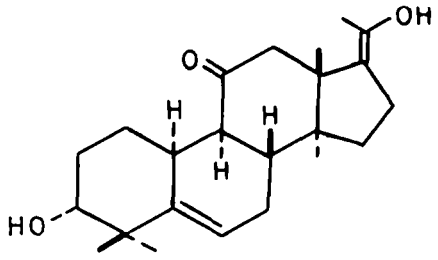
I



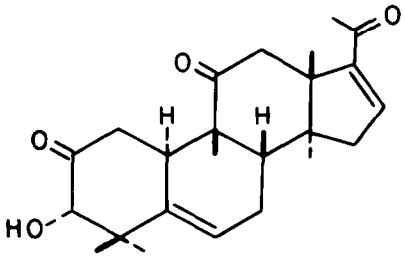
II : R = H
V : R = OOH
VI : R = OH



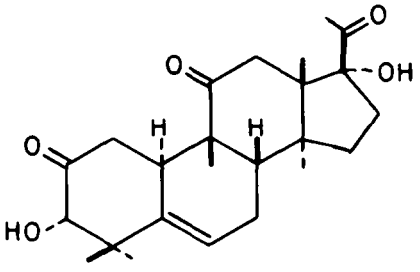
III



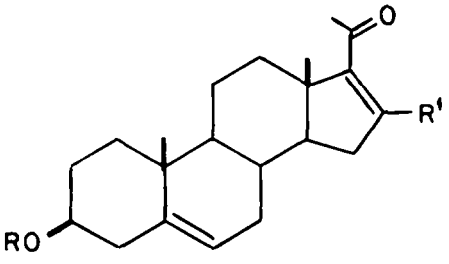
IV



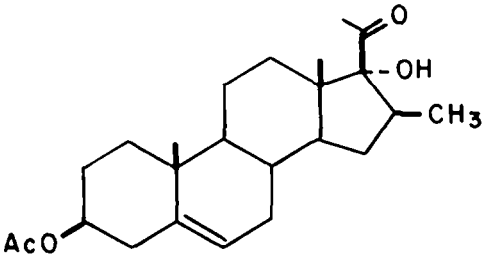
VII



VIII



IX : R = R¹ = H
X : R = Ac, R¹ = CH₃



XI

special challenge since other efficient methods[3, 6] of 17-hydroxylation of 20 ketones are carried out in the presence of strong base. A pronounced concentration effect was noticed here for the first time. In experiments using a concentration of 1 mg/ml in the hydrogenation step, a 70% reaction (GLC) was found whereas the yield dropped to 50% when a concentration of 2 mg/ml was employed. From the reaction mixture the main product was isolated and characterised as the 17α-hydroxy derivative (VIII). Its NMR spectra showed that no isomerisation of the α-ketol grouping in ring A occurred.

It was of special interest to establish whether the above method could also be used for the preparation of 17-hydroxy-20-oxo-steroids of the natural configuration. In various experiments with 3β-hydroxy-pregna-5,16-dien-20-one (IX) and its acetate, the yield of 17-hydroxy derivative was less than 5%. In contrast, 3β-acetoxy-16-methylpregna-5,16-dien-20-one (X) gave a 70% reaction (GLC) to give the 17α-hydroxy derivative (XI). The yield was appreciably lower (13%) when the free alcohol of IX was used. This may be due to the poor solubility of the hydrogenation product and/or preferential crystallisation of the 16,17-dihydro-20-ketone during evaporation of the ethanolic solution. A similar observation was made by Kohler and Thompson.[7]

A number of other steroidal enones were also studied. Under standard conditions no evidence of oxidation via a reactive intermediate enol was found for cholest-4-en-3-one, cholest-5-en-7-one and $\Delta^{9(11)}$-dehydrohecogenin. In all three of these compounds an intermediate enol formed by 1,4-addition of hydrogen, would be dialkyl substituted. In the case of cholest-5-en-4-one and cholest-4-en-6-one, the intermediate enolic double bond would be trialkyl substituted. GLC evidence for some oxidation to 5α-hydroxy-cholestan-4-one was found with the former compound. The reaction on cholest-4-en-6-one was studied in detail and also found to be concentration dependent. A 60% yield of a mixture of 5-hydroxy derivatives (mainly 5α) was achieved when the reaction was carried out in a concentration of 1 mg/ml.

To explain the differences observed in reactivity for the "hydrogenation-oxidation" of the enones studied, two main factors must be distinguished. Firstly, the formation of a reactive enol is dependent on a 1,4-addition of hydrogen to the enone, and here structural and conformational factors will be important. These factors are discussed in detail in a paper by Augustine *et al.*[8] The second important factor is the relative stability of the enol once formed and the ease of its protonation with solvents to give the ketone. Steric and conformational factors will no doubt play an important role.

The reactivity of cholest-5-en-4-one and cholest-4-en-6-one is probably due to the fact that the intermediate enols are trialkylsubstituted. It is known[9] that neutral enols are stabilised by alkyl substituents as a result of their electron-releasing effect relative to hydrogen, and through hyperconjugation.

In the cucurbitacin derived Δ^{16}-20-ketones the presence of a 14α-Me group is expected to favour 1,4-, opposed to 1,2-addition of hydrogen. The difference in reactivity between the two enones (IX and X) is more difficult to explain. One possible explanation could be that 1,2-addition of hydrogen is preferred in the enone (IX) leading to an unreactive 16,17-dihydro-derivative whereas in X the extra methyl group at position 16 introduces a steric factor in the transition state leading to 1,2-, but not to 1,4-addition. Such a difference is, however, not very obvious from the inspection of space-filling models and another explanation for the difference in behaviour of these two steroids was explored.

It is possible that a 1,4-addition of hydrogen is favoured in both enones leading to 20-enols which differ in their relative stabilities. Models show that rapid protonation by solvent from the α-face of the enol from compound IX should present no problem and would lead to an unreactive product with a 17β-acetyl side chain. In the enol from compound X the attack of solvent will lead, presumably under kinetic control[5], to the thermodynamically unstable β-side chain. Attack from the α-face will be impaired by the bulky Me groups at positions 13 and 16. These factors may play a role in increasing the relative stability of the enol derived from compound X.

Recently Gardner *et al*[6] described a one-step procedure for the 17-hydroxylation of 20-ketones. The autoxidation was carried out at a low temperature in the presence of triethyl phospite which reduced the initially formed hydroperoxide *in situ*. This procedure was investigated in two experiments, differing only in the amount of triethyl phosphite added, using the 16,17-dihydro-20-ketone (II). Two chromatographically pure but non-crystalline products were isolated. A compound, M^+ 390, is probably the product of hydroxylation at positions 9 and 17 and is the major product in the experiment using the larger excess of triethyl phosphite. The major product from the experiment with less triethyl phosphite, was a 17-ketone (ν_{max} 1745 cm^{-1}). Its M^+ of 346 show that it is probably also oxygenated at position 9. The results are in accordance with the expectation that, once the 17-hydroperoxy group has been reduced (large excess of triethyl phosphite), the competing base-catalysed decomposition to a 17-ketone, will be arrested.

It is evident that a base-catalysed hydroxylation method cannot be used for a selective 17-hydroxylation of cucurbitacin derived 11,20-diketones and that the procedure described in this paper, of reacting neutral enols generated by hydrogenation, is at present the only one available.

EXPERIMENTAL

Unless otherwise specified, spectra were recorded as follows: IR spectra (Perkin–Elmer 237) for solns in $CHCl_3$, NMR spectra (Varian HA-100) for solns in $CDCl_3$ with TMS as internal standard, and mass spectra (A.E.I. MS-9). Optical rotations were determined for solns in $CHCl_3$ at 24° with a Bendix NPL automatic polarimeter. Analytical and preparative TLC were performed with Merck pre-coated silica gel plates-using $CHCl_3$ containing 1–5% MeOH. Products were analyzed with a Barber-Colman Model 10 Gas chromatograph using a 2 meter column of 1% SE-30 plus 1% QF_1 on Gas-chrom Q (60–80 mesh). At a column temp of 230° and argon flow rate of 250 ml/min the retention times for the 17-ketone (III), 20-ketone (IV) and 17α-hydroxy-20-ketone (V) were 4, 7 and 9·3 min, respectively.

Preparation of 5% Pd-$BaSO_4$ *catalyst*[10]. $PdCl_2$ (Merck, 1 g) was dissolved in N HCl (5 ml) and the clear soln diluted with water to 50 ml. A ppt of $BaSO_4$ was prepared by adding a soln of Na_2SO_4 (1·77 g) in water (25 ml) slowly to a magnetically stirred soln of $BaCl_2 \cdot 2H_2O$ (2·47 g) in water (25 ml) at 75°. The ppt was washed 8 times by decantation with hot water.

The ppt was suspended in water (50 ml) at 75° to which was added the above $PdCl_2$ soln (10 ml) and 35% formaldehyde aq (1·25 ml). The suspension was kept at 70–80° and N NaOH was added from a burette over a period of 30 min. The pH of the stirred suspension was continuously measured and after the addition of ca 4·1 ml of NaOH aq, a pH of 7·5 was reached. Stirring was continued for another 10 min during which time the pH showed a slow drift to lower values. An apparent partial separation of metal and support was often observed but on prolonged washing, the catalyst regained an even-grey appearance. The catalyst was washed (20 min stirring, 10 min settling) 10 times with water (100 ml). After standing overnight under water, it was given a final wash, collected on a filter and dried in a desiccator over silica gel to give 2·2 g of catalyst practically free from traces of acid or base.

Standard hydrogenation and autoxidation procedure. Hydrogenations were carried out in a Gallenkamp Micro Hydrogenation Apparatus HR-100 under atm press at 20°. The enone (10–20 mg) was hydrogenated in 96% EtOH (distilled from KOH and Zn through a short fractionation column) using 5% Pd-$BaSO_4$ catalyst (20 mg) (1 mole absorbed in 20–60 min). The catalyst was removed by filtration through kieselguhr (cleaned by extensive washing with water and EtOH) and the solvent was evaporated *in vacuo* at a temp below 30°. The product was dissolved in benzene (2 ml) and shaken in air at room temp. GLC analysis showed the autoxidation to be complete in 2–5 min. Reduction of the resultant hydroperoxide to the corresponding hydroxy compound was performed by the addition of an excess of triethyl phosphite (20% soln in benzene). The usual work-up involved washing with water to remove triethyl phosphate, drying over Na_2SO_4, evaporation of the solvent and brief drying at 100° *in vacuo*.

3α,17α-*Dihydroxy*-4,4,14α-*trimethyl*-10α-5-*ene*-11,20-*dione* (VI). The crude product, prepared under standard conditions, showed a 90% conversion (GLC) to a mixture of the two isomeric 17-hydroxy

derivates. Separation on a silica plate gave two products in a ratio of ca 5:1. The less polar major component (GLC retention time 9·3 min) was crystallised from $CHCl_3$-MeOH to give the 17α-*hydroxy*-20-*ketone* (VI), m.p. 247–248°, $[\alpha]_D$ −149° (*c*, 0·58). (Found: C, 73·9; H, 9·3; M^+, 374·2453. $C_{23}H_{34}O_4$ requires: C, 73·8; H, 9·2%; M, 374·2457).

The more polar minor component (GLC retention time 9·6 min) gave a mass spectrum (M^+ 374) strongly resembling that of the major component.

3α-*Hydroxy*-4,4,14α-*trimethyl*-10α-*androst*-5-*ene*-11,17-*dione* (III). The reaction mixture from the hydrogenation of I (100 mg) was evaporated *in vacuo* at 20° and dissolved in toluene (5 ml). The soln was shaken in air and after 10 min, GLC (peak at 4 min) showed the autoxidation to be complete. The 17-hydroperoxide was decomposed by heating the above soln under reflux. The course of the reaction was followed by GLC of samples after the addition of triethyl phosphite. After 5 hr no further reduction of the 17-ketone occurred indicating the completion of the reaction and an 85% yield (GLC) of the 17-ketone.

The crude product contained some II which was removed by chromatography on a sheet (46 × 57 cm) of Whatman No. 3MM filter paper, impregnated with formamide (30%) using 3:1 hexane-EtOAc for development. The position of the 17-ketone (R_f 0·6) was revealed on cut-off strips with a vanillin-phosphoric acid spray reagent.[2] Extraction of the paper with $CHCl_3$—MeOH, washing with water to remove formamide and crystallisation from $CHCl_3$—Et_2O, gave the 17-*ketone* (III), m.p. 217–219°, $[\alpha]_D$ −102° (*c*, 0·57), ν_{max} 1740 cm^{-1}. (Found: C, 76·3; H, 9·2. $C_{21}H_{30}O_3$ requires: C, 76·3; H, 9·2%).

3α,17α-*Dihydroxy*-4,4,14α-*trimethyl*-19(10→9β)*abeo*-10α-*pregn*-5-*ene*-2,11,20-*trione* (VIII). The hydrogenation product of VII[1] was autoxidised and the resulting hydroperoxide reduced under standard conditions to give a crude product which showed a GLC peak at 14 min (62% of area) for the main 17-hydroxy derivative. Separation on a silica gel plate and crystallisation from $CHCl_3$—MeOH gave the 17α-*hydroxy*-20-*ketone* (VIII), m.p. 234–238°, $[\alpha]_D$ +195° (*c*, 0·39), NMR singlet at δ 3·85 (C_3—H). (Found: C, 71·3; H, 8·8; M^+, 402·2417. $C_{24}H_{34}O_5$ requires: C, 71·6; H, 8·5%; M, 402·2406).

3β-*Acetoxy*-17α-*hydroxy*-16β-*methylpregn*-5-*en*-20-*one* (XI). Preparation of the 17-hydroxy-20-oxo derivative under standard conditions gave a product which showed a GLC peak (71% of area) at 9·7 min (column temp 218°). Purification by chromatography on a silica gel plate and crystallisation from $CHCl_3$—MeOH gave XI, m.p. 168–169°, $[\alpha]_D$ −55° (*c*, 0·27). (Found: C, 74·2; H, 9·4. $C_{24}H_{36}O_4$ requires: C, 74·2; H, 9·3%) (lit.,[11] m.p. 168–170°).

5α-*Hydroxy*-*cholestan*-6-*one and* 5β-*hydroxy*-*cholestan*-6-*one*. A preparation under standard conditions from cholest-4-en-6-one gave a product which showed 2 spots on TLC ($CHCl_3$) both more polar than either 5α- or 5β-cholestan-6-one. Separation on a silica gel plate gave 5α-hydroxy-cholestan-6-one, m.p. and mixed m.p. 150–153° as major component and 5β-hydroxy-cholestan-6-one, m.p. and mixed m.p. 103–104° as the minor component. The two products were further identified by their IR spectra. On GLC the 5α-hydroxy derivative was clearly separated (retention time of 9 min) from other products and a yield of 55% could be estimated. The total oxidation yield must be higher since the peak of the 5β-isomer coincided with that of the cholestan-6-one peaks.

3α-*Hydroxy*-4,4,14α-*trimethyl*-19-*nor*-10α-*pregn*-5-*ene*-11,20-*dione* (II). Compound I[2] (0·8 g) in 96% EtOH (200 ml) containing N. NaOH (2 ml), was hydrogenated at room temp in the presence of 5% Pd-$CaCO_3$ catalyst (uptake of one mole H_2 in 30 min). The catalyst was removed by filtration through kieselguhr and the filtrate neutralised with AcOH. The crude crystalline product showed no sign of oxidation when dissolved in benzene soln and was crystallised from $CHCl_3$—Et_2O to give II (640 mg), m.p. 196–198° (lit.,[2] m.p. 197–201°).

Oxygenations in the presence of strong base and triethyl phosphite. Sodium hydride (44 mg) was dissolved in t-BuOH (0·4 ml) and DMF (1·5 ml) containing triethyl phosphite (0·1 ml in the first experiment, 0·2 ml in the second experiment). The mixture was cooled to −20° and a soln of II (200 mg) in THF (0·2 ml) was added. O_2 was passed through the soln and the course of the reaction monitored by GLC. After one hr the reaction mixture was diluted with $CHCl_3$ and washed with water. The final products showed 2 major peaks at retention times of 4·5 and 9·5 min. A peak at 7·5 min, which was initially formed, rapidly decreased with time in both experiments.

The crude products from the two experiments were purified separately by repeated chromatography on silica gel plates and Whatman No. 3MM paper impregnated with formamide (30%) using 1:1 EtOAc-hexane as solvent. Two chromatographically pure but non-crystalline fractions, both probably mixtures of isomers, were isolated. The major product from the experiment with the larger excess of triethyl phosphite was the component with retention time 9·5 min. Its M^+ 390 showed the incorporation of 2 O atoms, most probably at positions 9 and 17 of the starting material.

From the experiment with less triethyl phosphite the major product with retention time of 4·5 min was isolated. It had M^+ 346 and IR bands at 1745 and 1708 cm^{-1} and must correspond to the a 17-ketone with an extra hydroxy-group (presumably at position 9).

Acknowledgements—The author wishes to express thanks to Mr. H. H. Lachmann, Mrs. A. Schultz and Miss K. Weiss for invaluable technical assistance.

REFERENCES

[1] J. R. Bull and K. Barbara Norton, *J. Chem. Soc.* (*C*), 1592 (1970)
[2] W. T. de Kock, P. R. Enslin, K. B. Norton, D. H. R. Barton, B. Sklarz and A. A. Bothner-By, *Ibid.* 3828 (1963)
[3] E. J. Bailey, D. H. R. Barton, J. Elks and J. F. Templeton, *Ibid.* 1578 (1962)
[4] J. B. Siddall, G. V. Baddeley and J. A. Edwards, *Chem & Ind.* 25 (1966)
[5] J. Attenburrow, J. E. Connett, W. Graham, J. F. Oughton, A. C. Ritchie and P. A. Wilkinson, *J. Chem. Soc.* 4547 (1961)
[6] J. N. Gardner, F. E. Carlon and O. Gnoj, *J. Org. Chem.* **33**, 3294 (1968)
[7] E. P. Kohler and R. B. Thompson, *J. Am. Chem. Soc.* **59**, 887 (1937)
[8] R. L. Augustine, D. C. Migliorini, R. E. Foscante, C. S. Sodona and M. J. Sisbarro, *J. Org. Chem.* **34**, 1075 (1969)
[9] D. N. Kirk and M. P. Hartshorn, *Steroid Reaction Mechanisms* p. 155. Elsevier, Amsterdam (1968)
[10] E. Mossetig and R. Mozingo, *Org. Reactions* Vol IV, p. 368 (1948)
[11] E. Shapiro, T. Legatt, L. Weber, M. Steinberg, A. Watnick, M. Eisler, M. G. Hennessey, C. T. Coniglio, W. Charney and E. P. Oliveto, *J. Med. Pharm. Chem.* **5**, 975 (1962)