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AN UNSTABLE HYDROXYMETHYL INTERMEDIATE FORMED IN THE METABOLISM OF 3-(4-CHLOROPHENYL)-1-METHYLUREA IN COTTON*

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Key Word Index—*Gossypium*; Malvaceae; metabolism of 3-(4-chlorophenyl)-1-methylurea; formation of the 1-hydroxymethyl derivative.

Abstract—An unstable metabolite was observed following incubation of 3-(4-chlorophenyl)-1-methylurea in an *in vitro* microsomal oxidase system isolated from etiolated cotton hypocotyl tissue. Its decomposition products were identified as formaldehyde and 4-chlorophenylurea. A previously characterized glucoside conjugate isolated from the metabolism of the same substrate in excised cotton leaves was found to be transformed into a stable, less polar product upon storage in anhydrous MeOH. This transformation product was identified by IR and MS data as 3-(4-chlorophenyl)-1-methoxymethylurea. The unstable microsomal metabolite, in turn, was also stabilized by derivatization with MeOH, and this derivative was characterized as 3-(4-chlorophenyl)-1-methoxymethylurea by isotopic dilution analysis; the original metabolite is therefore 3-(4-chlorophenyl)-1-hydroxymethylurea.

INTRODUCTION

A MIXED function oxidase system which *N*-demethylates 3-(phenyl)-1-methylureas was recently prepared from cotton leaf.[1,2] This system was found in the microsomal fraction after differential centrifugation and was shown to require molecular oxygen and reduced pyridine nucleotides as cofactors. The overall reaction for the enzymatic *N*-demethylation of 3-(4-chlorophenyl)-1-methylurea is given below:

The final products of reaction were identified as formaldehyde and 4-chlorophenylurea, and equal molar quantities of the two compounds were produced. However, during the isolation and characterization of the reaction products, an unstable metabolite was detected by TLC and autoradiography. Its identification is now reported.

RESULTS AND DISCUSSION

Preliminary Observations of an Unstable Metabolite

Initial evidence indicating that an unstable intermediate was involved in the microsomal *N*-demethylation of 3-(4-chlorophenyl)-1-methylurea was found during TLC[3] of the demethylation products. A radioactive spot of weak intensity was observed which migrated

* Part III in the series "*N*-Demethylation of Substituted 3-(Phenyl)-1-Methylureas". For Part II see *Phytochem.* **11**, 1919 (1972).

[1] D. S. FREAR, H. R. SWANSON and F. S. TANAKA, *Phytochem.* **8**, 2157 (1969).

[2] D. S. FREAR, *Science* **162**, 674 (1968).

[3] C. R. SWANSON and H. R. SWANSON, *Weed Sci.* **16**, 137 (1968).

just below 4-chlorophenylurea (Table 1). If the carbonyl-labeled substrate was used for preparation of this unstable metabolite, after isolating and rechromatographing this material, the mobility of the compound was found to be altered. The material now had the same R_f as 4-chlorophenylurea. However, if the methyl-labeled substrate was used, the radioactivity was lost from the isolated and rechromatographed material, which was observed below 4-chlorophenylurea. Furthermore, upon allowing the unstable metabolic product derived from the methyl-^{14}C substrate to stand at room temp. overnight or at 5° for an extended period of time, approx. 60–70% of the unstable metabolite was transformed to a more stable, less polar product when stored either in anhydrous MeOH or dry $CHCl_3$. When stored in MeOH, the transformation product had R_f (× 100) of 32 and 50 in solvents 1 and 2 (Table 1), respectively. On the other hand, if the material was stored in $CHCl_3$, R_f (× 100) were 50 and 71. Since the metabolite derived from the methyl-^{14}C substrate did not lose radioactivity during this transformation, there was no loss of the original methyl carbon in this process. Furthermore, the same transformation products were also observed when carbonyl-^{14}C substrate was used. These transformation products were stable and could be rechromatographed, whereas the initial metabolite was completely decomposed on TLC plates when left for 1–2 hr.

TABLE 1. TLC DATA OF SOME SUBSTITUTED PHENYLUREA ANALOGS*

Compound	R_f values (× 100) in: Solvent 1†	Solvent 2‡	Solvent 3§
3-(4-Chlorophenyl)-1-hydroxymethylurea	11	22	7
4-Chlorophenylurea	15	24	13
3-(4-Chlorophenyl)-1-methylurea	33	50	32
3-(4-Chlorophenyl)-1-methoxymethylurea	32	50	34
3-(4-Chlorophenyl)-1-ethoxymethylurea	50	71	42
3-(4-Chlorophenyl)-1,1-dimethylurea	50	52	57

* Thin-layer plates were prepared of silica gel HF (250 μ thickness).
† Benzene–acetone (2:1).
‡ Isopropyl ether–acetone (7:3).
§ $CHCl_3$–MeOH (20:1).

After isolation of the unstable intermediate derived from carbonyl-^{14}C substrate and further treatment with acid, the radioactive decomposition product was characterized as 4-chlorophenylurea by isotopic dilution analysis. Upon treatment of the unstable intermediate from methyl-^{14}C substrate with dilute acid, the radioactive decomposition product was identified as formaldehyde by previously described methods.[1] The fact that 4-chlorophenylurea and formaldehyde were the only decomposition products suggested that the unstable metabolite was probably 3-(4-chlorophenyl)-1-hydroxymethylurea. Furthermore, the reaction of urea with formaldehyde has been known to afford *N*-(hydroxymethyl)urea or methylolurea[4] as product.

Attempts were then made to derivatize the hydroxyl group in order to stabilize the molecule. Such derivatizing agents as (—)-menthyl chloroformate, *N*-heptafluorobuturylimidazole, bis(trimethylsilyl)-trifluoroacetamide, trifluoroacetic anhydride, phenylisocyanate,

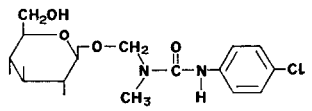
[4] C. R. NOLLER, *Chemistry of Organic Compounds*, p. 313, Sanders, Philadelphia (1957).

N,O-bis(trimethylsilyl)-acetamide, *N*-trimethyl-silylimidazole and trimethylchlorosilane were used, but none of these reagents produced a satisfactory derivative.

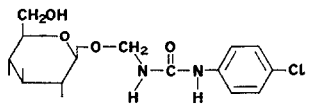
Using the method of Balba *et al.*,[6] benzyloxymethylisocyanate was prepared and further reaction with 4-chloroaniline yielded 3-(4-chlorophenyl)-1-benzyloxymethylurea. However, reductive cleavage for a 2-min period yielded only 3-(4-chlorophenyl)-1-methylurea. This, dehydration of the hydroxymethyl analog was followed by further hydrogenation to yield the isolated product. Even when the reduction time was reduced to 15 sec and the reaction mixture chilled, the same product was isolated. Thus, synthesis of 3-(4-chlorophenyl)-1-hydroxymethylurea by this method was not possible.

Characterization of Glucoside II *Transformation Product*

Important evidence to support the contention that the unstable metabolic product might be 3-(4-chlorophenyl)-1-hydroxymethylurea was the recent report of the β-D-glucosides of the *N*-hydroxymethyl analogs of 3-(4-chlorophenyl)-1,1-dimethylurea and 3-(4-chlorophenyl)-1-methylurea. These two glucosides I and II were formed from their respective parent substrates in excised cotton leaves.



(I)



(II)

While Glucoside I was reasonably stable, Glucoside II was relatively unstable. Purified II stored in anhydrous methanol was transformed into a stable, less polar product. Furthermore, transformation of methyl-^{14}C labeled II showed that the methyl carbon was retained even after loss of the glucose portion of the molecule. MS of this transformation product gave a parent ion at *m/e* 214, indicating that methanol had been substituted for glucose in the original metabolite. After heating the transformation product with excess EtOH, a new product was isolated by TLC and MS of it afforded a molecular ion at *m/e* 228. This indicates that MeOH was eliminated and EtOH added, i.e. suggesting that the transformation product, resulting from the MeOH treatment of II, was 3-(4-chlorophenyl)-1-methoxymethylurea.

An authentic sample of 3-(4-chlorophenyl)-1-methoxymethylurea was prepared and its IR spectrum was identical to that of the transformation product resulting from reaction of II with MeOH. MS of both compounds showed identical fragmentation patterns with a molecular ion at *m/e* 214. Therefore, these data confirmed the identity of the transformation product of II in MeOH as 3-(4-chlorophenyl)-1-methoxymethylurea.

Identification of the Unstable Hydroxymethyl Metabolite

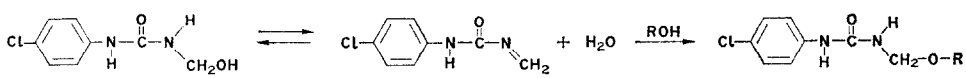
In preliminary studies, the unstable metabolic product derived from microsomal preparations was transformed into a more stable, less polar product, as was II when stored in MeOH at room temp. In order to characterize this unstable metabolite, it was necessary to determine if its transformation product upon treatment with MeOH was identical to the 3-(4-chlorophenyl)-1-methoxymethylurea identified from transformation of Glucoside II with methanol.

[5] J. A. Durden, Jr., H. W. Stollings, J. E. Casida and M. Slade. *J. Agric. Food Chem.* **18,** 460 (1970).
[6] M. H. Balba, M. S. Singer, M. Slade and J. E. Casida. *J. Agric. Food Chem.* **16,** 841 (1968).

For confirmation, the material was isolated from the microsomal preparation by chromatography on a Sephadex LH20 column and stored in the $CHCl_3$–MeOH eluate to prepare the transformation product. However, after isolation, the unstable metabolite was found to be slightly contaminated with the parent substrate, 3-(4-chlorophenyl)-1-methylurea. Following conversion to the transformation product in MeOH, separation of the product from the parent substrate by TLC was not possible (see Table 1). In order to obtain a pure sample, the unstable intermediate was eluted from a chromatographic column with $CHCl_3$–EtOH, and the isolated metabolite was allowed to stand in this solvent to form the ethyl analog of the transformation product. Purification by TLC was now easily achieved. The solubility of the ethyl analog was considerably greater in EtOH than the methyl analog in MeOH; consequently, precipitation of the ethyl analog resulted in considerable loss of material so that isotopic dilution analysis with this compound was not feasible. Therefore, the ethyl analog was converted to the methyl analog by heating the material in anhydrous MeOH at 50–55° for 3 days; it was characterized as 3-(4-chlorophenyl)-1-methoxymethylurea by isotopic dilution analysis.

DISCUSSION

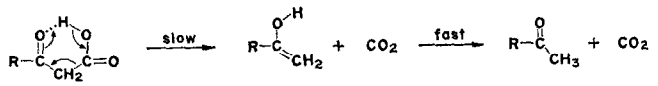
From the present identification of the hydroxymethyl metabolite, an explanation of some of the anomalous results obtained earlier in our studies is possible. It is now clear that the early transformation product from storage of the hydroxymethyl metabolite in MeOH is 3-(4-chlorophenyl)-1-methoxymethylurea formed by reaction with the MeOH, and the second transformation product observed when the hydroxymethyl metabolite was stored in $CHCl_3$ is 3-(4-chlorophenyl)-1-ethoxymethylurea formed by reaction with the EtOH (1% conc.) used as a preservative in the $CHCl_3$. From the data obtained, the apparent mechanism for reaction of the hydroxymethyl metabolite with alcohol appears to be:



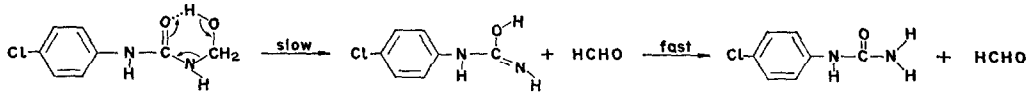
This reaction was found to take place only under anhydrous conditions. If trace quantities of water were present, the material would either remain as the hydroxymethyl metabolite or decompose to formaldehyde and 4-chlorophenylurea. An equilibrium between the hydroxymethyl metabolite and the proposed dehydrated intermediate may exist in this reaction. This is supported by the fact that the hydroxymethyl metabolite was readily attacked by alcohol at room temp. only under anhydrous conditions, and heat was required to transform the methoxymethyl product into the ethoxymethyl analog. The equilibrium concentration would be expected to strongly favor the hydroxymethyl intermediate. At this time the existence of the dehydrated intermediate has not been fully demonstrated; however, indirect evidence suggests that this intermediate is involved in the reactions observed for 3-(4-chlorophenyl)-1-hydroxymethylurea as well as its alcohol derivatives. Observations supporting a dehydrated intermediate are: (a) The relative ease of hydrogenolysis of 3-(4-chlorophenyl)-1-benzyloxymethylurea to 3-(4-chlorophenyl)-1-methylurea indicates that dehydration of the hydroxymethyl product had occurred subsequent to further hydrogenation to yield the final isolated product; (b) the conversion of the hydroxymethyl metabolite to the methoxymethyl and subsequently to the ethoxymethyl analog required the elimination of a molecule of water and subsequently methanol; (c) glucoside I was incapable of elimina-

tion of glucose; consequently, preparation of the methoxymethyl analog of this metabolic conjugate was not possible. On the other hand, Glucoside II easily formed the methoxymethyl analog by addition of methanol after the elimination of glucose; (d) the MS of the benzyloxymethyl, ethoxymethyl, and methoxymethyl analogs of 4-chlorophenylurea all showed a strong peak at *m/e* 182 which is the calculated molecular weight of the dehydration product.

The mechanism for dealkylation of 3-(4-chlorophenyl)-1-hydroxymethylurea may be very similar to the mechanism suggested for the decarboxylation of β-keto acids.[7] In the mechanism for the decarboxylation of β-keto acids, intramolecular hydrogen bonding leads to the formation of a cyclic 6-membered transition state. With the partial transfer of the carboxyl proton, the ease of heterolytic carbon to carbon bond cleavage is improved; hence, an overall increase in the rate of decarboxylation over that expected if there were no participation of the keto group in the decarboxylation reaction.



The facile demethylation of the hydroxymethyl metabolite upon standing on a thin-layer plate or in a nonreactive solvent at room temperature is probably due to the participation of the carbonyl group in the dealkylation reaction. Similar to the β-keto acids, there may be a partial transfer of the hydroxyl proton through intramolecular hydrogen bonding in the transition state leading to enhancement of the rate of dealkylation. Thus, in the proposed mechanism for the demethylation reaction of 3-(4-chlorophenyl)-1-hydroxymethylurea, the proton is transferred to the carbonyl oxygen by a cyclic 6-membered transition state and formaldehyde is eliminated.

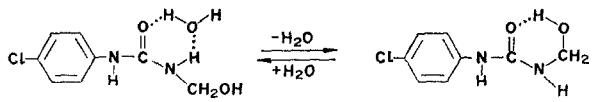


Although a hydroxymethyl intermediate is probably involved in the metabolic dealkylation of 3-(4-chlorophenyl)-1,1-dimethylurea, this metabolite was not observed. The substituted dimethylurea is apparently dealkylated by the same reaction mechanism as the substituted monomethylurea, since the isolation and identification of I and II from excised cotton leaves indicates that the dimethyl and monomethyl substrates are metabolized by the same pathway. However, the formation of glucose conjugation products by microsomal preparations has not been demonstrated. Without any means of stabilization, the hydroxymethyl intermediate of 3-(4-chlorophenyl)-1,1-dimethylurea is probably formed and immediately dealkylated to yield 3-(4-chlorophenyl)-1-methylurea, the product isolated from the metabolic reaction. Currently, there is no evidence available to explain the greater instability of the hydroxymethyl intermediate of the dimethyl substrate over that derived from the monomethyl substrate.

A possible explanation for the greater stability of the hydroxymethyl intermediate derived from the monomethyl substrate may be that the hydroxymethyl group can be held in a configuration *anti* to the carbonyl group by hydrogen bonding[7] with the water that is present in the metabolic reaction. This configuration would allow the proton that is bonded

[7] E. S. GOULD, *Mechanism and Structure in Organic Chemistry*, pp. 29, 346, Holt, New York (1959).

to nitrogen to be situated *syn* to the carbonyl group which, in turn, would reduce the probability that the molecule would enter into the transition state for the dealkylation reaction.



Considering the case of the hydroxymethyl intermediate derived from the dimethyl substrate, there appears to be no steric preference for the hydroxymethyl group in either the *syn* or *anti* configuration with respect to the carbonyl group. Furthermore, the possibility for solvent participation in stabilization of the molecule by means of hydrogen bonding would not be possible since a proton is not available on nitrogen. Consequently, the unstable transition state involving intramolecular hydrogen bonding to the carbonyl group would be favored. With the participation of the carbonyl group in the dealkylation step, the hydroxymethyl intermediate derived from the dimethyl substrate would be readily dealkylated to afford the monomethyl metabolite. Under our experimental conditions we observed only the demethylated product, indicating that the hydroxymethyl intermediate of the dimethyl substrate was too unstable to be detected under these experimental conditions.

EXPERIMENTAL

Plant material and preparation of microsomal fraction. Etiolated cotton plants (*Gossypium hirsutum* L.) of the Stoneville 213 variety were grown in growth chambers at 32° and 70% relative humidity in subirrigated vermiculite for 4–6 days. Etiolated hypocotyl tissues were prepared as previously described for the isolation of the microsomal fraction.[1] The microsomal fraction was sedimented at 78 000 *g* for 80 min after removal of larger particular matter by centrifugation at 1500 *g* for 10 min and at 17 500 *g* for 20 min.[2]

Enzymatic reaction. The metabolic products of 3-(4-chlorophenyl)-1-methylurea obtained from microsomal *N*-demethylase activity were generated by the method previously reported. Substrates used in this study were the carbonyl labeled-^{14}C and the methyl labeled-^{14}C 3-(4-chlorophenyl)-1-methylurea. For the isolation of the unstable metabolic product, the enzymatic reaction was increased about 10×, and the incubation period was extended to 60 min. The enzymatic reaction was terminated by rapid freezing in dry ice-acetone, and the reaction mixture was dried by lyophilization prior to extraction.

Isolation of the unstable metabolic product. The lyophilized reaction mixture was extracted with three 5-ml portions of MeOH, and the combined extracts were reduced to dryness on a rotary vacuum evaporator. The extract was dissolved in *ca.* 2 ml of $CHCl_3$ and added to a 1·5 × 15 cm column of Sephadex LH20. Elution of the column was first carried out with $CHCl_3$ until the starting substrate, 3-(4-chlorophenyl)-1-methylurea, was eluted (*ca.* 150 ml). Then the solvent was changed to $CHCl_3$–MeOH (10:1) for the elution of the hydroxymethyl intermediate (*ca.* 50 ml). The eluate was immediately reduced to dryness at room temp. under vacuum, and the residue was dissolved in acetone. Development of a TLC of this material confirmed that the isolated radioactive material was the unstable hydroxymethyl metabolite.

Isotopic dilution analysis of 4-chlorophenylurea. The unstable metabolite was prepared by incubating carbonyl-^{14}C 3-(4-chlorophenyl)-1-methylurea (sp. act. of 5·85 mCi/mM) in the microsomal preparation. The unstable metabolic product was isolated by TLC, and treated directly on the TLC plate with 0·1 M acetate buffer of 4·5 pH. The plate was allowed to stand at room temp. for 24 hr to ensure complete decomposition of the metabolite. After treatment, this material was rechromatographed, and the product was isolated from the TLC plate. Approx. 2·60 × 10^5 dpm of material was isolated. To the radioactive material was added 200 mg of nonradioactive 4-chlorophenylurea as carrier. This material was recrystallized 7× from acetone, and a constant specific activity of 1·31 × 10^3 dpm/mg was achieved for the final three recrystallizations.

Identification of the transformation product of glucoside II. After isolation of II by means of Sephadex G10, the product was stored in anhydrous MeOH. Upon analysis of this material by TLC in solvent 1, the glucoside was found to be transformed into a different product with a R_f of 32. To further charaterize this product obtained from the glucoside, the material was purified by TLC, and analyzed by IR and MS. IR, (KBr) 3325 (NH), 1625 (C=O), 1130 and 1095 (doublet, C–N–C–O–C), 1068, 908, 830, 508 cm^{-1}; MS, *m/e* 214 (molecular ion), 182 (M-32), 153 (M-61), 127 (M-87). With the initial loss of 32 from the molecular ion, it appeared that MeOH was being readily eliminated from this compound. This result suggested that the derivative of II was capable of alcohol exchange. To determine if alcohol exchange could take place, the

derivative (*m/e*, 214) was heated in absolute EtOH at 45° for 2·5 days. Examination by TLC showed that a different derivative was obtained. This material was purified by TLC and analyzed by MS; MS, *m/e* 228 (molecular ion), 182 (M-46), 153 (M-75), 127 (M-101). From the MS data, it became apparent that MeOH was substituted for glucose upon storage of II in MeOH (*m/e*, 214), and that EtOH was substituted for MeOH upon heating the MeOH derivative in EtOH (*m/e*, 228). Therefore, authentic samples of 3-(4-chlorophenyl)-1-methoxymethylurea and 3-(4-chlorophenyl)-1-ethoxymethylurea were prepared in the laboratory for direct comparison. Both the methyl and ethyl analogs of II were found to be identical to the synthesized material in all respects when compared by TLC (cochromatography), IR, and MS.

Characterization of the unstable metabolic product. The unstable metabolic product was found to be stabilized in the crude plant extract and could be stored at −10° for an extended period. After purification the stability of the compound was reduced, although storage at −10° in acetone for several days was still possible. Purification of the unstable metabolite was carried out by elution from a Sephadex LH20 column with $HCCl_3$–MeOH (10:1), and the metabolite was allowed to stand in the eluate overnight at room temp. to yield a stable MeOH derivative. After conversion to this methoxymethyl analog, however, purification by TLC was not possible since the mobility of the methoxymethyl derivative was nearly identical to that of the contaminant (trace of parent substrate) in all solvents utilized. Therefore, the EtOH derivative of the unstable metabolite was prepared by elution of the Sephadex LH20 column with $CHCl_3$–EtOH (10:1), and the isolated metabolite was allowed to stand at room temp. in the eluate overnight. Following transformation, the purification of the product was easily achieved by TLC. The ethoxymethyl analog was heated in anhydrous MeOH at 50–55° for 3 days to convert this derivative into the methoxymethyl analog for the isotopic dilution experiment. The methodology for the conversion of 3-(4-chlorophenyl)-1-ethoxymethylurea to 3-(4-chlorophenyl)-1-methoxymethylurea was verified by trial experiments using an authentic sample of the ethoxymethyl analog. Approx 90–95% conversion was achieved, and the identity of the conversion product from the trial experiments was confirmed by TLC, IR, and NMR. For the isotopic dilution analysis, approx. $8{\cdot}4 \times 10^4$ dpm (sp. act. of 5·85 mCi/mM) of the MeOH derivative was isolated and purified by TLC. To the radioactive material was added 500 mg of nonradioactive 3-(4-chlorophenyl)-1-methoxymethylurea as carrier. This material was recrystallized 6× from anhydrous MeOH to afford a product with a constant specific activity of $1{\cdot}65 \times 10^2$ dpm/mg for the final three recrystallizations.

Synthesis of 3-(4-*chlorophenyl*)-1-*benzyloxymethylurea.* Benzyloxymethyl isocyanate was prepared according to the method of Balba *et al.*[6] by means of their acid azide route. For the preparation of 3-(4-chlorophenyl)-1-benzyloxymethylurea, benzyloxymethyl isocyanate (3·26 g, 0·02 mol) and 4-chloroaniline (2·35 g, 0·02 mol) were refluxed in anhydrous benzene (50 ml) for 18 hr with protection from atmospheric moisture. The solvent was evaporated under reduced pressure, and a homogenous product was realized by recrystallization from acetone. m.p.,[8] 134–136°; NMR, (acetone-d_6, 37°, Me_4 Si internal reference) 4·57 (2 H, singlet, $-C\underline{H}_2-$phenyl group), 4·78 (2 H, doublet, $J = 7$ Hz, becomes singlet on D_2O treatment, $N-C\underline{H}_2-$group), 7·13–7·58 ppm [9 H, 2 doublets (7·18 and 7·48 ppm) of the *para* substituted phenyl ring overlapped by the 5 proton peak at 7·30 ppm of the monosubstituted phenyl ring, $J = 9$ Hz, 4-chlorophenyl group]; IR; (KBr) 3340 (NH), 1660 (C=O), 1115 and 1095 (doublet, C–N–C–O–C), 919, 900, 825, 738, 696, 505 cm^{-1}; MS, *m/e*, 290 (molecular ion, very weak intensity), 182 ($M-C_6H_5CH_2OH$), 153 ($M-C_6H_5CH_2OCH_2NH_2$), 127 ($M-C_6H_5CH_2OCH_2N{=}C{=}O$). *Anal.*[9] Calcd. for $C_{15}H_{15}O_2N_2Cl$: C, 61·96; H, 5·20; O, 11·01; N, 9·63. Found: C, 61·40; H, 5·41; O, 11·47; N, 9·76.

Synthesis of 3-(4-*chlorophenyl*)-1-*methoxymethylurea.* The 3-(4-chlorophenyl)-1-benzyloxymethylurea was dissolved in an excess of anhydrous methanol and refluxed for 24 hr. Conversion to 3-(4-chlorophenyl)-1-methoxymethylurea was almost quantitative. After reaction the solvent was evaporated under vacuum and a pure product was obtained by recrystallization from MeOH. m.p.,[8] 168–170°; NMR, (acetone-d_6) 3·25 (3 H, singlet, $-OC\underline{H}_3$ group), 4·61 (2 H, broad singlet, becomes a sharp singlet on D_2O treatment, $-C\underline{H}_2-$group), 7·18 and 7·48 ppm (4 H, 2 doublets, $J = 9$ Hz, 4-chlorophenyl group); IR, (KBr) 3325 (NH), 1625 (C=O), 1130 and 1095 (doublet, C–N–C–O–C, this portion of spectrum appears very much like an acetal),[10] 908 cm^{-1} (strong band not observed in the monomethyl analog); MS, *m/e*, 214 (molecular ion), 182 ($M-CH_3OH$), 153 ($M-CH_3OCH_2NH_2$), 127 ($M-CH_3OCH_2N{=}C{=}O$). *Anal.*[9] Calcd. for $C_9H_{11}O_2N_2Cl$: C, 50·36; H, 5·17; O, 14·91. Found: C, 49·91; H, 5·22; O, 15·16.

Synthesis of 3-(4-*chlorophenyl*)-1-*ethoxymethylurea.* Initial attempts for synthesis were carried out by refluxing a solution of 3-(4-chlorophenyl)-1-benzyloxymethylurea in absolute EtOH. However, the conversion rate was rather slow with yields of about 20, 40 and 50% after reflux times of 1, 2 and 3 days, respectively. Thus, the preparation of 3-(4-chlorophenyl)-1-ethoxymethylurea was carried out by dissolving 3-(4-chlorophenyl)-1-methoxymethylurea in an excess of absolute EtOH and heating atre flux for 18 hr. By this method, the conversion to the ethoxymethyl analog was approximately quantitative. The product was purified by

[8] Melting point measurements were carried out on a Thomas–Hoover melting point apparatus, and all melting points were reported uncorrected.

[9] Elemental analyses were performed by the Huffman Laboratories, Inc., Wheatridge, Colorado.

[10] The splitting of the C–O–C–O–C bonds of 1,1-dibutoxyethane is observed at 1095 and 1132 cm^{-1}. The *Sadtler Standard Spectra*, No. 13946, The Sadtler Research Laboratories, Philadelphia (1966).

recrystallization from EtOH, m.p.,[8] 152°; NMR, (acetone-d_6) 1·10 (3 H, triplet, $J = 7$ Hz, $-CH_3$ group), 3·50 (2 H, quartet, $J = 7$ Hz, $-O-CH_2-Me$ group), 4·65 (2 H, doublet, $J = 7$ Hz, becomes a singlet on D_2O treatment, $N-CH_2-$ group), 7·18 and 7·48 ppm (4 H, 2 doublets, $J = 9$ Hz, 4-chlorophenyl group); IR, (KBr) 3330 (NH), 1640 (C=O), 1100 and 1075 (doublet, C–N–C–O–C), 1018, 834 and 510 cm^{-1}; MS, *m/e*, 228 (molecular ion, weak intensity), 182 ($M-CH_3CH_2OH$), 153 ($M-CH_3CH_2OCH_2NH_2$), 127 ($M-CH_3CH_2-OCH_2N=C=O$). *Anal.*[9] Calcd. for $C_{10}H_{13}O_2N_2Cl$: C, 52·50; H, 5·73. Found: C, 51·63; H, 5·73.

Attempted catalytic hydrogenolysis of 3-(4-*chlorophenyl*)-1-*benzyloxymethylurea.* The reduction was achieved by means of a Parr low pressure hydrogenation apparatus, series 3910. Hydrogenolysis was carried out with 1·16 g (0·004 mol) of 3-(4-chlorophenyl)-1-benzyloxymethylurea dissolved in 50 ml of dry tetrahydrofuran (distilled over $LiAlH_4$) using 3 g of 10% Pd on charcoal[11] as catalyst. The reaction vessel was flushed free of air using N_2 and filled to a pressure of 15 lb with H_2. Reduction times of 2 min or 15 sec were used. The product was isolated by filtering the reaction mixture and evaporating the solvent from the filtrate under reduced pressure. Examination by TLC indicated that the major hydrogenolysis product in both the 2 min and the 15 sec reductions was 3-(4-chlorophenyl)-1-methylurea and not the desired product, 3-(4-chlorophenyl)-1-hydroxymethylurea.

[11] R. MOZINGO, in *Organic Syntheses* (edited by E. C. HORNING), Vol. 111, p. 687, Wiley, New York (1955).