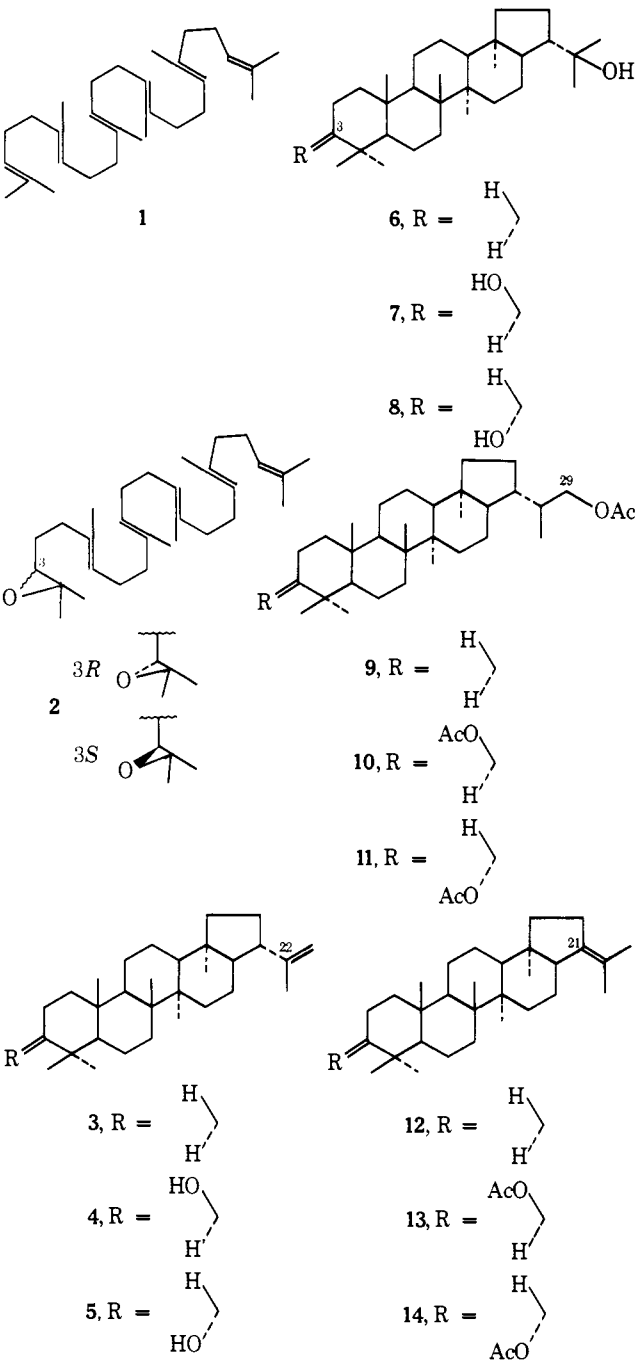

This demonstrates the expected incorporation of squalene into hopane derivatives; the yield of conversion (certainly not the best attainable) is ca. 6%: 3% into each of the products.

When [12,13-^{3}H]-(3*R*,*S*)-oxidosqualene **2** was incubated with the same enzyme system under the same conditions, as expected, radioactive cycloartenol and lanosterol were not detected. This is in accord with the absence of detectable amounts of sterols in *Acetobacter*. However, *unexpectedly, four new labeled metabolites*, absent from the normal lipid fraction of *Acetobacter*, were isolated. These were characterized as the 3-hydroxyhopane derivatives **4, 5, 7,** and **8** by recrystallization to constant specific radioactivity, with authentic samples. The products **4** and **5** were acetylated, and compounds **7** and **8** were converted to **13** and **14** obtained by dehydration of the monoacetates of **7** and **8**. The respective products retained their specific radioactivity at a constant level on multiple recrystallizations. The acetates of **4** and **5** were converted into the 29-acetoxy derivatives **10** and **11** and crystallized to constant specific radioactivity.

This proves that the enzyme system responsible, in *A. rancens*, for the cyclization of squalene itself is also able to cyclize the unnatural substrate, squalene epoxide, to give products differing from the normal ones only by the additional presence of a 3-hydroxy group. Furthermore, whereas eucaryotic cells cyclize only (3*S*)-squalene epoxide and leave the (3*R*) enantiomer intact,[5] the enzyme system of *A. rancens* forms 3β-hydroxyhopane derivatives from the (3*S*) enantiomer, and their 3α-isomers from the (3*R*) enantiomer.[6] The conversion of **2** is ca. 3% into the 3β- and 2% into the 3α-epimers.

The prokaryotic squalene cyclase of *Acetobacter rancens* is therefore "primitive" in that it displays a remarkable lack of substrate specificity at the site of initiation (acidic) of the cyclization. This indicates that the absence of 3β-hydroxytriterpenes in this microorganism (and in many others)[7] is due to the absence of squalene epoxide.[8]

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(8) We thank the CNRS (RCP No. 282, LA No. 31 and ERA No. 487) for support and Professor P. Benveniste for the provision of facilities and helpful discussions.
(9) Address correspondence to this author at Institut de Chimie.

Claude Anding, Michel Rohmer, Guy Ourisson*[9]
Institut de Chimie and Institut de Botanique
Université Louis Pasteur
Strasbourg, France
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Base-Promoted Hydrolysis of Amides at Ambient Temperatures

Sir:

Often one is presented with the problem of hydrolyzing amides which are acid sensitive and thermally labile. The obvious method for use in such situations would be hydrolysis with base. However, base hydrolysis of amides is frequently an inefficient process. While addition of hydroxide ion to an amide of general formula **1** should occur with relative ease to produce **2,** the thermodynamically preferred route for the breakdown of **2** involves loss of hydroxide to regenerate **1.**[1-3] In principle, if the hydroxylic proton of **2** could be removed by a second strong base, **3** should be generated.[4,5] Cleavage of **3** with loss of amide anion would generate **4** and **5.** We now wish to report that we have developed reaction conditions which promote this transformation at room temperature.

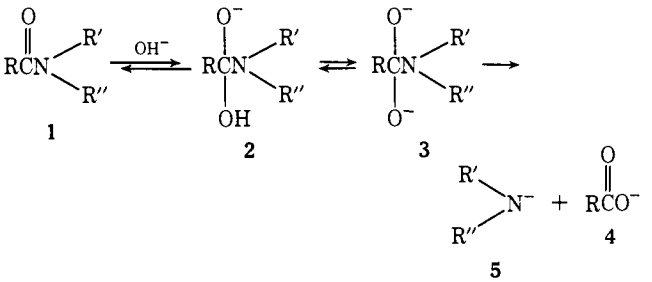
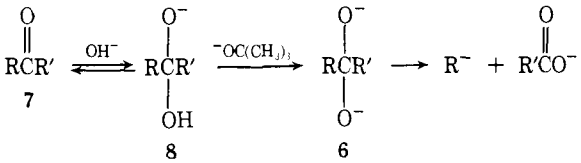


Table I. Yields Obtained in the Hydrolysis of Amides with Potassium *tert*-Butoxide–Water[a]

Amide	Amine	% yield	Acid	% yield	Time, h
$C_6H_5CON(CH_3)_2$	$(CH_3)_2NH$	85[b]	$C_6H_5CO_2H$	96	5
$CH_3CON(CH_3)C_6H_5$	$C_6H_5NHCH_3$	98	CH_3CO_2H	96[c]	3
$C_6H_5CONC_5H_{10}$	Piperidine	93[b]	$C_6H_5CO_2H$	96	48
$HCON(C_6H_5)_2$	$(C_6H_5)_2NH$	100	HCO_2H	55[d]	2
$CH_3CON(CH_3)_2$	$(CH_3)_2NH$	65[b]	CH_3CO_2H	88[c]	12
$(CH_3)_3CCON(CH_3)_2$	$(CH_3)_2NH$	82[b]	$(CH_3)_3CCO_2H$	88	27[e]

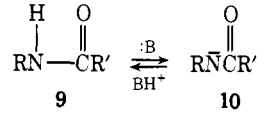
[a] All hydrolyses were carried out at room temperature in diethyl ether unless otherwise indicated. [b] Isolated as the *p*-toluenesulfonamide. [c] Acetic acid was determined titrimetrically after neutralization with Amberlite IR-120 ion-exchange resin. Analysis of standard sodium acetate by this method routinely gave 90–92% values. The values quoted in the table have been corrected for this tendency of the method to give values which are 9% low. [d] Isolated as the phenylhydrazide. [e] The hydrolysis was carried out in refluxing tetrahydrofuran.

In considering the requirement that **3** be an intermediate in the hydrolysis process, we were drawn to the analogous intermediacy of **6** in the cleavage of nonenolizable ketones by hydroxylic base.[6] The best conditions for this cleavage involved treatment of 1 equiv of the ketone in ether with the basic system derived from ca. 6 equiv of potassium *tert*-butoxide and 2 equiv of water in ether. Reaction of the water with the potassium *tert*-butoxide generated finely divided, essentially anhydrous potassium hydroxide (2 equiv) and *tert*-butyl alcohol (2 equiv). The strongly nucleophilic and poorly solvated hydroxide added to the carbonyl of **7** to produce **8.** The conversion of **8** into the dianion **6** was accomplished by the excess potassium *tert*-butoxide. Once **6** was generated, cleavage occurred with ease.[7] Thus, we felt that conditions which were applicable to the base-promoted cleavage of nonenolizable ketones should be sufficient to readily hydrolyze amides.



In a typical procedure, a slurry of potassium *tert*-butoxide (13.7 g, 0.122 M), water (0.67 g, 0.037 M), and the amide (0.0185 M) in diethyl ether was stirred vigorously at room temperature (ca. 24°), and the course of the reaction was followed by TLC analysis. When the amide was completely gone, the reaction was cooled in an ice bath and ice was added to the reaction mixture until two layers formed. The layers were separated and the products were isolated from the appropriate layers. Table I lists the yields of both the amine and acid fragments obtained in a series of hydrolyses. As can be noted from the table, the yields are excellent in those cases where the products do not require special methods of analysis. Both aliphatic and aromatic amides can be hydrolyzed at relatively low temperatures. Only in the case of the highly hindered *N,N*-dimethylpivalamide did the reaction require heating.

All of the examples listed in Table I are tertiary amides. This illustrates both a strength and a weakness of the method. Both secondary and primary amides are extremely resistant to hydrolysis under our conditions. For instance, acetamide was hydrolyzed to the extent of less than 10% after 45 h. Acetanilide showed no detectable hydrolysis products after 7 days. Presumably, this is due to the conversion of **9** into **10** in an acid–base reaction. This means that our method will allow the selective hydrolysis of a tertiary amide in the presence of a secondary or primary amide. In this regard, it offers an attractive counterpart to the procedure of White[8] for the cleavage of secondary amides in the presence of tertiary amides.



We believe that the ease with which tertiary amides can be hydrolyzed by our method offers certain distinct advantages relative to the protecting of secondary amines as amides. In principle, any secondary amine which has been protected via conversion to a tertiary benzamide derivative can be regenerated at room temperature under basic conditions.

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Paul G. Gassman,* Philip K. G. Hodgson, Robert J. Balchunis
Department of Chemistry, University of Minnesota
Minneapolis, Minnesota 55455
Received November 14, 1975

Magnetic Resonance Spectroscopy on Carbon-13 Labeled Uracil in Transfer Ribonucleic Acid[1]

Sir:

We would like to report results on the incorporation of carbon-13 uracil, labeled at the C-4 position, into transfer ribonucleic acid (tRNA) using a mutant strain of *Salmonella typhimurium.* The labeled bulk tRNA's were studied with carbon-13 nuclear magnetic resonance spectroscopy (^{13}C NMR).[2] The carbon-13 labeled uracil was prepared from ^{13}C enriched potassium cyanide. The strain of *S. typhimurium,* designated JL-1055,[3] has the genotype *pyrA, pyrG, cdd,* and *udp.* The bacteria were grown in 10-l. batches at 37 °C in a minimal media of glucose and salts, plus arginine, cytidine, and carbon-13 labeled uracil, these additional nutrients being required by JL-1055 for normal growth. The cells, harvested in the late log phase, were recovered by centrifugation and the tRNA was extracted[4] and purified by DEAE column chromatography.[5] The puri-