Application of Chemical Reactions on Thin-layer Chromatoplates. IV. Triterpenes

A. M. Osman, M. El-Garby Younes,* and Mrs. F. M. Ata
Department of Chemistry, Faculty of Science, University of Assiut, Assiut, Egypt, A.R.E.

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Some specific reactions of triterpenoids had been performed on thin-layer chromatoplates. The reactions conducted were: oxidation with oxygen in K-*t*-butoxide, benzilic acid rearrangement, decarboxylation with lead tetraacetate, Wolff-Kischner reduction, reduction with sodium borohydride, reduction with lithium aluminium hydride, oxidation with chromium trioxide in pyridine and in acetic acid, oxidation with manganese dioxide in chloroform, methylene chloride and isopropyl alcohol, insertion of an oxygen atom in ring A of triterpenoids by the formation of acid anhydride and reduction by a modified Clemmensen method.

Previously,[1)] we had carried out some chemical reactions characteristic of triterpenoids on thin-layer chromatoplates. Since we are working mainly on compounds belonging to this group, we decided to do more specific chemical reactions of triterpenes on thin-layer chromatoplates in order to serve in widenning the aspects of the technique and to overcome some difficulties encountered in our work on natural products. Besides the reactions carried out with amino-acids[2)] on chromatoplates, also we had perfomed reactions characteristic of the monoterpenes.[3)] Really, we found that the application of chemical reactions on chromatoplates solved several problems, particularly in the field of natural products.

Generally speaking, the reaction is carried out by mixing the substance under examination mostly in solution form, with the reactants within the area of a small spot at the starting line of a plate of an inert adsorbent. The mixture of reactants is then subjected to the favourable conditions and suitable length of time to simulate, as far as possible, the normal reaction conditions in the flask. The adsorbed layer is then treated in the normal manner as a chromatographic medium for the resolution of the components of the reaction mixture. Thus, development with a suitable solvent system enables the detection of the formed derivative or comparison with the expected material, spotted at the starting line immediately prior to development. Differentiation of the derivative from the starting material, if any remains, is always possible if the appropriate conditions are used.

Results and Discussion

Reactions involving oxidation of the triterpene ketones with oxygen in potassium *t*-butoxide at room temperature[4)] to give the corresponding diosphenol, proceeded with difficulty, and most of the starting material was obtained unreacted (compound **1**, **2**, *cf.* Table 1), while the resulting diosphenol was obtained in a poor yield.

Benzilic acid rearrangement[5)] of the diosphenol, was performed on the chromatoplate successfully for all the compounds tried, and the resulting α-hydroxy acids were obtained in a reasonable yield (**3**—**4** *cf.* Table 1).

Reactions of the α-hydroxy acids with lead tetraacetate in acetic acid[6)] at room temperature was almost complete and smooth and the A-nor triterpenes were obtained in good yields.

Wolff-Kischner reduction of the triterpene ketones, proceeded under severe conditions and with difficulty, and the reacted materials were obtained in very poor yields, while most of the starting ketone remained unchanged.

Reduction of compounds (**8**—**10**, *cf.* Table 1) with sodium borohydride was performed easily with a good yield, while reduction with a suspension of lithium aluminium hydride in dry ether proceeded with difficulty and was incomplete, even by changing the experimental conditions,[3)] for instance prolonging the time of exposure period.

Oxidation of compounds (**13**—**15** in Table 1) with chromium trioxide–pyridine at room temperature overnight was difficult, while oxidation with chromium trioxide–acetic acid at 50 °C for a few minutes proceeded smoothly with a good yield.

Oxidation of the hydroxy triterpenes (**13**—**18**; *cf.* Table 1) with manganese dioxide in different solvents[7)] gave interesting reuslts and the yield changes according to the nature of the solvent. Using chloroform as a solvent the yield was poor, while by using methylene dichloride and isopropyl alcohol the oxidation reaction gave a better yield.

The formation of the triterpene anhydride[8)] from the secodiacid (**19**; *cf.* Table 1) by heating at 100 °C for 2 hr with acetic anhydride proceeded with difficulty and a poor yield of the desired anhydride was formed, while most of the starting material was obtained unchanged.

Reduction of the keto-groups to methylene groups by a modified Clemmensen procedure[9)] was proceeded smoothly under mild conditions using active zinc dust and acetic anhydride saturated with hydrogen chloride gas at 0 °C. The resulting methylene compounds were obtained in good yields (See experimental for compounds **8** and **20** in Table 1).

Conclusion: The results obtained in this study show that the application of chemical reactions of the triterpenoids on chromatoplates can afford reliable informations regarding the "preparation" of known derivatives and determining whether a given reaction can take place, all with the use of only negligible amounts of material.

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* To whom inquires should be addressed.

Table 1

No. of compound	Starting material (R_f)	Reaction product (R_f)	Solvent system[a]
	Oxidation with oxygen in K-*t*-butoxide		
1	Methyl 20(29)-dihydrobetulonate (0.68)	The corresponding diosphenol (0.43)	I
2	Methyl oleanonate (0.82)	Diosphenol (0.32)	I
	Benzilic acid rearrangment		
3	Methyl 2-hydroxy-ketoolean-1,12-dien-28-oate (0.58)	Methyl 2β-hydroxy-2α-carboxy-A-nor-olean-12-en-oate (0.09)	II
4	Methyl 2-hydroxy-3-keto-20(29)-dihydrolup-1-en-28-oate (0.57)	Methyl 2β-hydroxy-2α-carboxy-A-nor-20(29)-dihydrolup-28-oate (0.11)	II
	Decarboxylation with lead tetraacetate		
5	Methyl 2β-hydroxy-2α-carboxy-A-nor-olean-12-en-28-oate (0.09)	Methyl 2-keto-A-nor-olean-12-en-28-oate (0.63)	II
6	Methyl 2β-hydroxy-2α-carboxy-A-nor-20(29)-dihydrolup-28-oate (0.11)	Methyl 2-keto-A-nor-20(29)-dihydrolup-28-oate (0.57)	II
	Wolff-Kischner reduction		
7	Methyl 2-keto-A-nor-olean-12-en-28-oate (0.9)	A-Nor-olean-12-en-28-oic acid (0.64)	III
8	β-Amyrenone (0.18)	β-Amyrene (0.93)	IV
9	Methyl oleanonate (0.51)	Olean-12-en-28-oic acid (0.14)	II
	Reduction with sodium borohydride		
9	Methyl oleanonate (0.51)	Methyl oleanolate (0.27)	I
8	β-Amyrenone (0.94)	β-Amyrin (0.65)	V
10	Betulonic acid (0.92)	Betulinic acid (0.79)	V
	Reduction with lithium aluminium hydride		
11	Uvaol diacetate (0.93)	Uvaol (0.39)	V
12	Methyl 2α,3β-diacetoxy-urs-12-en-28-oate (0.8)	2α-Hydroxy-uvaol (0.3)	V
	Oxiation with CrO_3 and pyridine		
13	Methyl 2α-hydroxy-ursolate (0.3)	Diosphenol (0.8)	V
14	Eburicoic acid (0.17)	Did not react	
15	β-Amyrin (0.62)	β-Amyrenone (0.94)	V
	Oxidation with CrO_3 and acetic acid		
14	Eburicoic acid (0.12)	Corresponing ketone (0.73)	VI
13	Methyl 2α-hydroxy-ursolate (0.3)	Diosphenol (0.8)	V
	Oxidation with MnO_2 in chloroform		
15	β-Amyrin (0.31)	β-Amyrenone (0.61)	II
16	Lupeol (0.26)	Lupenone (0.62)	II
	Oxidation with MnO_2 in methylene dichloride		
17	Methyl oleanolate (0.16)	Methyl oleanonate (0.76)	I
18	Methyl maslinate (0.14)	Diosphenol (0.8)	V
	Oxidation with MnO_2 in isopropyl alcohol		
14	Eburicoic acid (0.16)	Corresponding ketone (0.94)	V
13	Methyl 2α-hydroxy-ursolate (0.3)	Diosphenol (0.8)	V
	Insertion of an oxygen atom in ring A		
19	2,3-Seco-urs-12-en-2,3,28-trioic acid 28-methyl ester (0.68)	The corresponding anhydride (0.96)	VII
	Modified Clemmensen reduction		
20	Euphone (0.41)	Euphadiene (0.95)	VIII
8	β-Amyrenone (0.17)	β-Amyrene (0.96)	IX

a) Solvent system: I:benzene–ether (9: 1), II: benzene, III: light petroleum–benzene (1: 1), IV: light petroleum–benzene (4: 1), V: benzene–ether (4: 1), VI: light petroleum–benzene (1: 4), VII: benzene–ether (1: 1), VIII: light petroleum–benzene (3: 2), IX: light petroleum–benzene (3: 1).

Experimental

Chromatoplates. These were prepared by slurring Kiesel gel G. (Merck) in water (1: 2 by weight) and applying manually onto plates (5×15 cm), followed by heating at 120 °C for 2 hr, then placed in a drying desiccator containing calcium chloride for drying. The development of the plates was done by placing them for 15 min in a tank containing iodine.

*Oxidation with Oxygen in K-*t*-Butoxide.* This reaction was performed by treating the ketonic triterpene spot in *t*-butyl-alcohol at the starting line of the chromatoplate with a saturated solution of potassium *t*-butoxide. The plate was then placed in a vacuum desiccator having a saturated atmosphere of *t*-butyl alcohol, the plate was evacuated from air and a stream of oxygen was then passed through for 2 hr. At the end of the reaction, the plate was dried in air prior to development.

Benzilic Acid Rearrangement. This reaction of the diospenols **3** and **4** (*cf.* Table 1) was achieved by treating the diosphenol spot in ethanol at the starting line of a chromatoplate with ethanolic KOH (10%). The plate was placed in a desiccator containing a saturated solution of the reagents and then heated at 70 °C for 5 hr after which the plate was cooled in air before chromatographic development.

Decarboxylation with Lead Tetraacetate. This reaction was performed by treating the α-hydroxy acid spot with a suspension of lead tetraacetate in acetic acid, the spotting of the reagent was continued for 10 min. The plate was placed in a desiccator containing acetic acid and the whole was maintained at 45 °C for 1 hr. After this period the spot was treated with ethylene glycol to destroy the excess of lead tetraacetate. The plate was dried and ready for chromatographic development.

Wolff-Kischner Reduction. This reaction was conducted on thin-layer chromatoplates by treating the triterpene ketonic spot under examination in ethanol with hydrazine hydrate (95%) and ethylene glycol. The plate was placed in a desiccator containing ethyl alcohol and ethyleneglycol, then the whole were heated at 150 °C in an oven for 2 hr, after this period the plate was cooled in air and a concentrated solution of ethanolic potassium hydroxide was applied on the reacting spot and heated again for extra 4 hr. At the end of this period the spot was cooled and acidified with sulfuric acid (1 M) prior to development.

Reduction with Sodium Borohydride. The ketone spot in methanol was treated with a suspension of sodium borohydride in water: methanol (5: 1) and the plate was heated as usual in the desiccator at 55 °C for 2 hr to give mainly the resultant alcohol after development.

Reduction with Lithium Aluminium Hydride. The reduction was performed by treating the ketone spot with a suspension of lithium aluminium hydride in dry ether at room temperature overnight prior to development.

Oxidation with Chromium Trioxide. *(A) In Pyridine*: The triterpene alcohol spot in pyridine was treated with chromium trioxide in pyridine and the plate was left in a desiccator containing pyridine for overnight. It showed that most of the starting alcohol remained unchanged.

(B) In Acetic Acid: The alcohol spot in acetic acid was treated with a solution of chromium trioxide in acetic acid and the chromatoplate in an atmosphere of acetic acid was heated at 50 °C for 15 min after which it was dried prior to development.

Oxidation with Manganese Dioxide. *(A) In Chloroform*: This reaction was performed by treating the triterpene alcohol spot n chloroform with manganese dioxide in chloroform and the plate was placed in a desiccator saturated with an atmosphere of chloroform and the whole was left at room temperature overnight after which the plate was dried in air prior to development.

(B) In Methylene Dichloride: The procedure is the same as that mentioned under method (A), except chloroform was replaced by methylene dichloride.

(C) In Isopropyl Alcohol: The reaction was performed as that under method (A), by replacing isopropyl alcohol instead of chloroform.

Formation of Acid Anhydride. The seco-diacid spot was treated with acetic anhydride and the plate was put in a desiccator containing acetic anhydride and the whole was heated at 100 °C for 2 hr, after which the plate was cooled prior to chromatographic development.

A Modified Clemmensen Reduction. This reaction was conducted on a tlc plate by treating the ketonic compound under examination at the starting line with a suspension of active zinc dust[9] in acetic anhydride. The plate was then placed in a vacuum desiccator containing a saturated atmosphere of acetic anhydride and the whole was cooled at 0 °C. Hydrogen chloride was passed into the desiccator until the space inside it became saturated with hydrogen chloride, then the whole system was left for 2 hr at 0 °C. The spot was treated with a solution of sodium carbonate and the plate was dried in air prior to development.

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