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Note

A note on oxide opening in withasteroids on solid surface†

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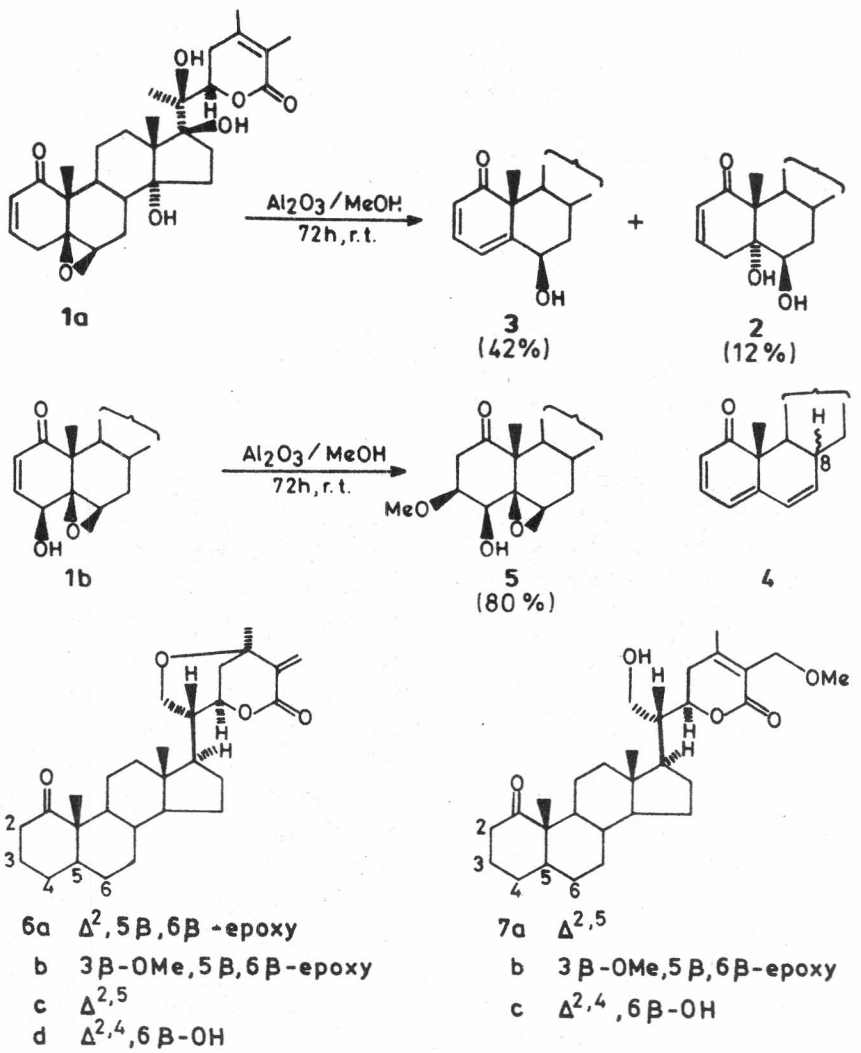
The effect of alumina on withasteroids having 5β, 6β-epoxy-2-en-1-one **1a**, 5β, 6β-epoxy-4β-hydroxy-2-en-1-one **1b** and cyclic allyl ether **6c** systems has been studied. Attempt has been made to rationalise the difference in the behaviour of **1a** and **1b.**

A wide variety of substitution pattern is discernible in the AB rings of withasteroids and simple methods for inter-conversion of withasteroids differing only in the substituents of their AB rings are well recorded[1]. While 5β, 6β-epoxy-2-en-1-one grouping, as is present in withanolide-E **1a**, is quite common in withasteroids, compounds like withanolide-S **2** and withaperuvin-C **3** having respectively, 5β, 6β-dihydroxy-2-en-1-one and 6β-hydroxy-2,4-dien-1-one moieties are also known, and their formation may very well be envisaged by opening of the epoxide ring of **1a**. Nitalla and Lavie[2] made an in-depth study of the opening of the steroidal 5β, 6β-epoxide group, particularly under thermal conditions on a solid support and obtained withaperuvin-C **3** as a minor product (16%) when withanolide-E **1a** was adsorbed on silica gel H powder and heated at 100°C for 24 hr. The major products of this reaction were two C-8 epimers of 2,4,6-trien-1-one **4**, formed apparently by the dehydration of **3** and subsequent epimerisation at the allylic site at C-8.

It has been observed by us that the conversion of **1a** to **3** takes place rather smoothly at ambient temperature on a bed of alumina and the yield is much higher than that recorded with silica gel at elevated temperature. When a methanolic solution of withanolide-E **1a** was adsorbed on a bed of alumina and left for 72 hr prior to desorption, the major product was withaperuvin-C **3** which was accompanied by a significant amount of withanolide-S **2** and nearly half of the unreacted material. Replacement of methanol by organic bases like morpholine or diethylamine for dissolution of **1a**, significantly improved the yield of **3** but failed to produce any Michael addition product, as can be expected[3] with the enone grouping of **1a**. This observation led to the logical assumption that the key step in the transformation of **1a** to **3** is the generation of a resonance stabilised carbanion at C-4, effected by abstraction of a proton from this active site by alumina and/or organic bases.

Unlike **1a**, however, 4β-hydroxy-withanolide-E **1b** yielded the Michael addition product **5** of methanol as the sole product on a bed of alumina. The difference in behaviour of the compounds **1a** and **1b** under identical reaction conditions is obviously due to the presence of a hydroxyl group at C-4 of the latter molecule. It is plausible that the difference in acid strength between the hydrogen of the hydroxyl group at C-4 and the allylic hydrogen at this site leads to preferential formation of alcoholate ion which, in turn, opposes the generation of carbanion at C-4 and thus favours normal Michael addition reaction. This explanation alone is not probably adequate as the presence of hydroxyl group at C-4 is not essential for Michael addition reaction in 5β, 6β-epoxywithasteroids. It was earlier observed by us[4] that withametelin-F[5] **6a** which has AB ring substituents same as that of withanolide-E **1a** but differs from the latter in side chain and its orientation, yields its methanol adduct, withafastuosin-C **6b,** albeit in low yield, on a bed of alumina. In fact this observation prompted us to verify the generality of this reaction but it was proved to be otherwise as we failed to get any methanol adduct of withanolide-E **1a** under the reaction condition. It is therefore, conjectured that the course of the reaction on alumina depends upon

†Part 30 in the series on withasteroids. For Part 27 see Manickam *et al., Phytochemistry*, 41, **1996**, 981. Part 28, *Nat Prod Sci* and Part 29, *Indian J Chem*, 35B, 1996, 1311.



the molecule as a whole and not merely on AB ring substituents.

Besides its action on steroidal 5β, 6β-epoxides, alumina was found to act on the oxide bridge of withametelin **6c**, a withanolide having a bicyclic side chain with an allyl ether system[6]. A methanolic solution of withametelin **6c** on standing on a bed of alumina for 72 hr was completely converted to secowithametelin **7a**, a compound that was earlier obtained by the treatment of a methanolic solution of **6c** with perchloric acid[6,7]. Besides withafastuosin-C **6b** and withametelin-B **6d**, the isolation of which was earlier reported[4], two other compounds could be isolated from the complex mixture obtained by the adsorption of a methanolic solution of withametelin-F **6a** on a bed of alumina and its desorption after 72 hr. The new compounds were characterised as **7b** and **7c**, formed respectively from **6b** and **6d** by 1,4-addition of methanol across the allyl ether bridge.

The transformations of withasteroids reported in this paper are interesting, and a better insight can be secured by subjecting more compounds under similar conditions. It is pertinent to mention in this connection that the transformations reported here could not be effected on silica gel column even on a much longer contact.

Experimental Section

General. Melting points were determined on a Toshniwal apparatus and are uncorrected. UV spectra were recorded on a JASCO UV-Vis 7800 instrument in MeOH. ^{1}H NMR spectra were recorded on a Brucker AMX 400 spectrometer in $CDCl_3$ or CD_3OD with Me_4Si as internal reference. Aluminium oxide, active neutral of Qualigens Fine

Chemicals was used. Silica gel (70-325 mesh) of Centron Research Laboratories and Silica gel G of Qualigens Fine Chemicals were used respectively, for CC and TLC.

All the known compounds were identified by direct comparison (mmp, Co-TLC, ^{1}H NMR, MS and UV) with authentic samples.

General procedure. A methanolic solution of the withasteroid (100 mg) was adsorbed on a small bed of alumina (5- g) and left at ambient temperature for 72 hr. The adsorbed material was then washed down with MeOH and the residue obtained on removal of solvent was chromatographed over a bed of silica gel.

Conversion of withanolide-E 1a to withanperuvin-C 3 and withanolide-S 2. The residue from MeOH washings of the alumina column loaded with **1a** was chromatographed over silica gel. Elution with C_6H_6-EtOAc (3:1) furnished the unreacted material (40 mg) and with C_6H_6-EtOAc (1:1) yielded a solid (42 mg) which was crystallised from Me_2CO to give colourless needles of **3**, mp 190-92°C (lit.[8], 190°C). Elution with EtOAc-MeOH (9:1) yielded a solid (12 mg) which crystallised from MeOH to give **2**, mp, 271-73°C (lit.[9], 272°C).

Repetition of the experiment using a solution of **1a** in diethylamine or morpholine and washing the alumina adsorbent with the same solvent yielded a non-basic residue (acid-insoluble and Dragendorff-negative). Chromatography of the residue over silica gel yielded **3** (80 mg) and **2** (7 mg).

Conversion of 4β-hydroxy-withanolide-E 1b to its methanol adduct 5. The desorbed material from the alumina bed loaded with **1b** was crystallised from MeOH to give fine needles (80 mg) of **5**, mp 185-86°C (lit.[10], 185°C).

Conversion of withametelin 6c to seco-withametelin 7a. The residue obtained by removal of solvent from MeOH washings of the column loaded with **6c** was chromatographed over silica gel. Elution of the column with hexane-EtOAc (3:1) yielded a homogeneous solid (70 mg) which crystallised from hexane-EtOAc mixture to give **7a** as white plates, mp 190-92°C (lit.[7], 190°C).

Conversion of withametelin-F 6a to withafastuosin-C 6b, withametelin-B 6d, 7b and 7c. The residue of the MeOH washings of the column loaded with **6a** was chromatographed over silica gel and eluted with solvents of increasing polarity. The homogeneous solid (10 mg) from hexane-EtOAc (3:1) eluate crystallised from the eluting solvent to give withafastuosin-C **6b**. mp 234-36°C (lit.[4], 235-37°C). Elution with hexane-EtOAc (1:1) yielded withametelin-B **6d** as microcrystalline white powder (7 mg); UV (MeOH): 314 nm, mp 283-86°C (lit.[11], 283-86°C). Elution with EtOAc yielded an amorphous powder (9 mg) which was characterised as **7b** from spectral analysis. Its FABMS (MH^+ at m/z 517, MNa^+ at m/z 539) indicated addition of two molecules of MeOH in **6a**; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.47 (1H, dt, *J*=13.3, 3.4 Hz, H-22), 4.27, 4.15 (1H, d, each, *J*=10.4 Hz, H_2-27), 4.04 (1H, br d, H_a-21), 3.75 (1H, dd, overlapped with a br m, H_b-21 and H-3), 3.37, 3.26 (3H, s each, 2 × OMe), 3.20 (1H, br s, H-6), 1.97 (3H, s, H_3-28), 1.15 (3H, s, H_3-19) and 0.68 (3H, s, H_3-18). Later fractions of EtOAc eluate yielded an amorphous solid (15 mg) which showed MH^+ peak at m/z 485, MNa^+ peak at m/z 507, indicating addition of only one molecule of MeOH in **6a** and its structure was clarified as **7c** from its spectral data. UV (MeOH): 312 nm; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.91 (1H, dd, *J*=9.6, 6.0 Hz, H-3), 6.14 (1H, d, 6.0, Hz, H-4), 6.01 (1H, dd, *J*=9.6, 0.6 Hz, H-2), 4.47 (1H, dt, *J*=13.3, 3.4 Hz, H-22), 4.57 (1H, br s, H-6), 4.27, 4.15 (1H, d, each, *J*=10.2 Hz, H_2-27), 4.04 (1H, br d, H_a-21), 3.75 (1H, dd, *J*=13.1, 3.1 Hz, H_b-21), 3.36 (3H, –OMe), 1.99 (3H, s, H_3-28), 1.47 (3H, s, H-19) and 0.80 (3H, s, H_3-18). This compound was also obtained as the sole product from withametelin-B **6d** under identical conditions.

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References

1 Ray A B & Gupta M, *Withasteroids, a growing group of naturally occurring steroidal lactones, in: Progress in the chemistry of organic natural products*, (Springer-Verlag, Wien, New York) **1994**, pp.1-106.

2 Nittala S & Lavie D, *J Chem Soc Perkin Trans-I*, **1982**, 2835.

3 Pelletier S W, Gebeyehu G & Mody N V, *Heterocycles*, 19, **1982**, 235.

4 Manickam M, Kumar S, Sinha-Bagchi A, Sinha S C & Ray A B, *J Indian Chem Soc*, 71, **1994**, 393.

5 Jahromi M A F, Manickam M, Gupta M, Oshima Y, Hatakeyama H & Ray A B, *J Chem Res (S)*, **1993**, 234.

6 Sinha S C, Kundu S, Maurya R, Ray A B, Oshima Y, Sinha Bagchi A & Hikino H, *Tetrahedron*, 45, **1989**, 2165.

7 Kundu A, Sinha S C, Bagchi A & Ray A B, Phytochemistry, **28**, **1989**, 1769.

8 Sahai M, Neogi P, Ray A B, Oshima Y & Hikino H, *Heterocycles*, 19, **1982**, 37.

9 Neogi P, Ph D Thesis, Banaras Hindu University, Varanasi, India, **1985**.

10 Neogi P, Sahai M & Ray A B, *Phytochemistry*, 26, **1987**, 243.

11 Gupta M, Bagchi A & Ray A B, *J Nat Prod*, 54, **1991**, 599.