Indian Journal of Chemistry
Vol. 41B, September 2002, pp. 1915-1918

# Chemical transformation of andrographolide†

Mahesh Pal*, Meenakshi Singh[1] & R P Sharma

Phytochemical Technology Division, Central Institute of Medicinal & Aromatic Plants P O CIMAP, Lucknow 226015, India
[1]Department of Chemistry, Isabella Thoburn P G College, Lucknow-226 001, India

*Received 1 April 1999; accepted (revised) 19 April 2002*

Tosylation of andrographolide **1**, with *p*-TsCl pyridine furnishes compounds **3** and **4**. The reaction of **1** with *in situ* generated nickel boride yields compounds **6** and **8**. Monotosylation of **6** followed by reaction with NaI refluxing in acetone furnishes the iodide **13**. But all attempts to generate an oxetane moiety in **13** corresponding to compund **2** failed.

Andrographolide **1** is known to possess hepatoprotective properties and its availability in excellent yield from the plant *Holmskioldia sanguinea*[1,2], (Verbenaceae) prompted us to carry out the chemical transformation on it in order to study the structure-activity relationship. Recently, a novel diterpene **2** has been isolated from the aerial parts of *Andrographis paniculata* which contains an oxetane moiety in it[3]. It has been established that the presence of oxetane moiety in the most potent anticancer drug taxol is essential for its biological activity[4]. It was therefore, of interest to us to synthesize compound **2** from andrographolide **1** by manipulation of the primary-secondary hydroxyl groups present at C-3 and C-19 respectively.

In order to selectively tosylate the primary hydroxyl group of andrographolide with *p*-toluene sulphonyl chloride in pyridine, the reaction was carried out at room temperature for 20 hr. when a mixture of two products was obtained which was separated by preparative TLC (hexane:ethyl acetate 85:15). The $^1$H NMR spectrum of less polar product suggested the presence of tosylate moiety in it. The mass spectrum did not show the molecular ion peak but the major peak at m/z 314 corresponding to $M^+$-$C_7H_8SO_3$ clearly indicated the formation of monotosylate. Therefore, structure **3** was assigned to the less polar product on the basis of spectral data.

The mass spectrum of more polar compound gave the molecular ion peak at m/z 332. In the $^1$H NMR spectrum, H-14 appeared as a broad singlet at 7.15 ppm, H-11 as a double doublet ($J$=16, 10 Hz.) at 6.40 ppm and H-12 as a doublet ($J$=16Hz) at 6.15 ppm. On the basis of above spectral data structure **4** was assigned to the more polar compound which was further characterized by making its diacetate **5**.

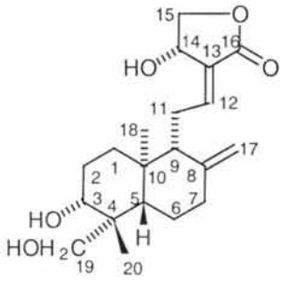
All attempts to generate the oxetane moiety from the hydroxyl at C-3 and the tosylate group at C-19 in compound **3** under various alkaline conditions where abortive as a mixture of products was formed in all the attempted reactions. It was then argued that this instability in compound **3** could probably be attributed to the presence of functionality at C-9. It was therefore, decided to carry out the transformation of andrographolide **1** to a more stable compound before attempting the formation of oxetane ring.

It has been reported that the hydroxyl group at the allylic position can be reductively removed by nickel boride generated *in situ* from sodium borohydride and nickel chloride[5]. Therefore, andrographolide **1** was treated with *in situ* generated nickel boride in DMF at room temperature. A mixture of two products was formed which was separated by preparative TLC (hexane: ethyl acetate 85:15).

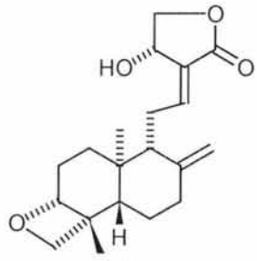
The less polar compound obtained as a gum showed a strong absorption band at 1761 cm$^{-1}$ indicating that the double bond at C-12, C-13 has undergone reduction to yield a saturated-γ-lactone. This was fully supported by $^1$H-NMR spectrum which indicated the absence of any signal below 5 ppm. The mass spectrum did not show the molecular ion peak but the major peaks at m/z 321($M^+$-$CH_3$) and 289 ($M^+$-$CH_3$-$CH_3OH$) suggested structure **6** for this compound. Acetylation of **6** with acetic anhydride and pyridine furnished the diacetate whose spectral data was in full accord with the assigned structure **7**.

The more polar compound obtained as an oil was identified as **8** on the basis of spectral data. The presence of hydroxyl group at C-14 in compound **8**

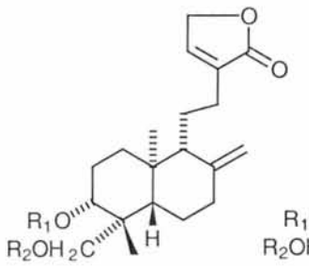
†CIMAP publication No.-98-20J

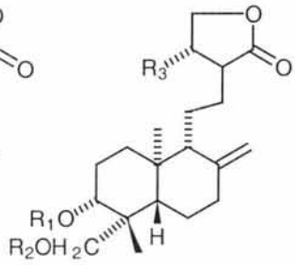


1

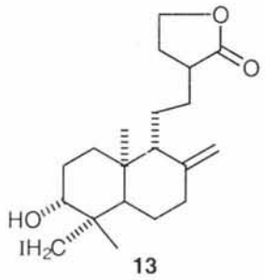


2





| | | | |
|---|---|---|---|
| **3** | $R_1$ = H, $R_2$ = Ts, $\Delta^{11,12}$ | **6** | $R_1$ , $R_2$, $R_3$ = H |
| **4** | $R_1$ , $R_2$ = H, $\Delta^{11,12}$ | **7** | $R_1$ , $R_2$ = Ac, $R_3$ = H |
| **5** | $R_1$ , $R_2$ = Ac, $\Delta^{11,12}$ | **8** | $R_1$ , $R_2$ = H, $R_3$ = OH |
| **9** | $R_1$ , $R_2$ = H | **12** | $R_1$ , $R_3$ = H, $R_2$ = Ts |
| **10** | $R_1$ , $R_2$ = Ac, | | |
| **11** | $R_1$ , $R_2$ = Ts | | |



13

prompted us to study its reaction with a base for confirming its structure as well as for obtaining a stable compound. Indeed, the reaction of compound **8** with sodium carbonate in methanol furnished quickly the α, β unsaturated-γ-lactone **9** in almost quantitative yield. Compound **9** was further characterized by converting it into the diacetate **10** whose spectral data was fully consistent with the assigned structure.

The reaction of **9** with *p*-toluene sulphonyl chloride in pyridine at room temperature furnished the monotosylate **11**. Reaction of **11** with sodium iodide in acetonitrile under refluxing condition led to extensive decomposition and no useful product could be isolated.

Reaction of **6** with *p*-toluene sulphonyl chloride in pyridine furnished the monotosylate **12** which on treatment with sodium iodide in acetonitrile under refluxing conditions provided the iodide **13**. All attempts to convert the iodide **13** into the corresponding oxetane product under various basic conditions were abortive due to the formation of a complex mixture of products in each case.

## Experimental Section

**Reaction of andrographolide 1 with *p*-toluene sulphonyl chloride in pyridine.** A solution of **1** (100mg) in dry pyridine (1 mL) was treated with *p*-TsCl (250 mg), stirred at room temperature for 20 hrs. to complete the reaction (TLC monitored). The reaction mixture was diluted with water (200 mL), extracted with ethyl acetate (4×50 mL), washed with water and dried. Solvent was evaporated and pyridine removed by co-distillation with toluene *in vacuo*. A mixture of two spot was obtained which were separated by preparative TLC (hexane:ethyl acetate 85:15). The less polar product as a gum (60 mg; 43.5%) was identified as **3**; IR (KBr) 1730, 1570, 1390, 1160, 1030, 980, 750; $^1$H NMR (80 MHz.): 7.60 (d, *J*=8Hz, 2H), 7.30 (d, *J*=8Hz, 2H), 7.08 (sbr, H-14), 6.80 (dd, *J*=16, 10Hz, H-11), 6.00 (d, *J*=16Hz, H-12), 4.70 (sbr, H-15, H-17a), 3.50-4.50 (overlapping signals of H-3, H-17b and H-19), 2.30 (sbr, 3H), 1.10 (s, H-20), 0.70 (s, H-18). The mass spectrum did not show the molecular ion peak but other major peaks were at m/z 314 ($M^+$-$C_7H_8SO_3$), 285, 267, 228, 194, 175, 159, 130, 112, 83. The more polar gummy product (30 mg; 32%) was identified as **4**[6]; IR (KBr): 1740, 1630, 1370, 1070, 1020, 758; $^1$H NMR (400 MHz): 7.15 (sbr, H-14), 6.90 (dd, *J*=16, 10Hz, H-11), 6.15 (d, *J*=16 Hz, H-12), 4.80 (sbr, H-15 and H-17a), 4.54 (s, H-17b), 4.3 (d, *J*=12Hz, H-19a), 3.50 (H-3 and hydroxyl protons), 3.35 (d, *J*=12Hz, H-19b), 1.35 (s, H-20), 0.85 (s, H-18). The mass spectrum gave the molecular ion peak at m/z 332 and other major peaks were at 314, 301, 296, 289, 285, 267, 257, 195, 159.

**Acetylation of 4.** A solution of **4** (10 mg) in dry pyridine (1 mL.) was treated with acetic anhydride (1.5 mL) and kept at room temperature for 24 hr. Diluted the mixture with water (200 mL) and extracted with chloroform (3×75 mL). Usual work-up gave a gummy residue **5** (8 mg; 67%); $^1$H NMR (400 MHz): 7.17 (sbr, H-14), 6.92 (dd, *J* =16,10 Hz, H-11), 6.15

(d, *J*=16 Hz, H-12), 4.82 (sbr, H-15), 4.80 (s, H-17a), 4.60 (t, *J*=9Hz, H-3), 4.58 (sbr, H-17b), 4.38 (d, *J*=12Hz, H-19a), 4.15 (d, *J*=12Hz, H-19b), 2.07 (s, 3H), 2.05 (s, 3H), 1.08 (s, 3H), 0.30 (s, 3H).

**Reaction of andrographolide 1 with nickelboride.** A stirred solution of **1** (50 mg) in dry DMF (3 mL) was treated with nickel chloride hexahydrate (300 mg) and $NaBH_4$ (750 mg) was added portionwise. Reaction was monitored by TLC. Usual workup afforded a residue which showed two spots on TLC which were separated by PTLC (ethyl acetate: hexane, 85:15) to give less polar gummy residue **6** (30mg; 62.5%); IR (KBr): 3400, 1761, 1669, 1456, 1378, 1295, 1264, 1203, 1158, 1058, 1020, 894, 750: $^1$H NMR (400 MHz): 4.82 (s,H-17a), 4.50 (dbr, *J*=12Hz, H-15a), 4.30 (m, H-15b), 4.17 (s, H-17b) 4.15(d, *J*=13Hz, H-19a), 3.45 (t, *J*=9Hz, H-3), 3.30 (d, *J*=13Hz, H-19b), 1.27 (s, H-20), 0.65 (s, H-18). The molecular ion peak was not localized and other major peaks were at m/z 321 (M-15), 289(M-15-32), 278, 236, 220, 208, 188, 174, 162,135, 121, 109, 95, 81,67, 55. The less polar compound **8** obtained as a gum (16mg;33%); IR (KBr): 3400, 1771, 1667, 1450, 1387, 1260,1170, 1140, 1020, 986, 980, 666; $^1$H NMR; 4.85 (s, H-17a), 4.55 (m, H-15a), 4.30 (m, H-15b), 4.30 (s, H17b), 4.17 (d, *J*=12Hz, H-19 a), 3.50 (overlapping signals of H-14 and H-3), 3.30 (d, *J* =12 Hz, H-19b), 1.27 (s, H-20), 0.64 (s, H-18). No molecular ion peak was seen, other peaks were at m/z 285 (M-2$H_2O$-$CH_2OH$), 257, 233, 220, 214, 206, 192, 181,169, 149, 123, 95, 69.

**Acetylation of 6.** A solution of **6** (10 mg) in dry pyridine (1 mL) was treated with acetic anhydride (1.5 mL) and left at room temperature for 48 hr. Usual work-up afforded a gummy residue **7** (6mg; 48 %); IR (KBr);1771, 1736, 1455, 1376, 1230, 1153, 1028, 801; $^1$H NMR (300 MHz): 4.88 (s, H-17a), 4.00-4.70 (overlapping signals of H-3, H-15, H-19), 4.35 (s,H-17b), 2.04(s,6H), 1.25 (s,H -20), 0.72 (s,H-18). No molecular ion peak was seen other peaks at m/z 360 ($M^+$-$C_2H_4O_2$),300 ($M^+$-2$C_2H_4O_2$), 287, 285, 272,259,245, 226, 206, 193, 187, 173, 161, 149, 133, 119, 105, 83, 71, 57, 43.

**Reaction of 8 with sodium carbonate.** A solution of **8** (10 mg) in MeOH (2mL) was treated with $Na_2CO_3$ (100 mg). The reaction mixture was stirred at room temperature for 5 hr and monitored by TLC. Usual work-up afforded a gummy residue **9**[6] (6 mg; 63%). IR (KBr): 3400, 1724, 1600, 1446, 1288, 1130, 1075, 744; $^1$H NMR (300 MHz); 709 (sbr, H-14), 4.88 (s, H-17a), 4.77 (sbr, H-15a and H-15b), 4.59 (s,H-17b), 4.17 (d, *J*=12 Hz, H-19a), 3.44 (dd, *J*=8, 6Hz, H-3), 3.31 (d, *J*=12 Hz, H-19b), 1.25 (s,H-20), 0.63 (s,H-18). No molecular ion peak was seen, other peaks were at 284 ($M^+$-$H_2O$-$CH_3OH$), 275, 256, 239, 213, 205, 185, 167, 149, 143, 135, 129, 109, 97, 69, 57.

**Acetylation of 9.** A solution of **9** (10mg) in dry pyridine (0.1 mL) was treated with acetic anhydride (0.2 mL) and left overnight at room temperature. Usual work-up gave a residue **10** (8 mg; 64%). IR ($CHCl_3$): 1740, 1730, 1640,1445, 1200, 1100, 758; $^1$H NMR (400 MHz): 7.10 m (H-14), 4.80-3.60(overlapping signals of H-17, H-15, H-19 and H-3), 1.25 (s,H-20), 0.72 (s,H-18). No molecular ion peak was seen, other major peaks were at m/z 298 ($M^+$-2$C_2H_4O_2$), 223, 205, 189, 175, 165, 135, 121, 79.

**Preparation of the tosylate 11.** A solution of **9** (10mg) in dry pyridine (1 mL) was treated with *p*-toluenesulphonyl chloride (200 mg) and stirred at room temperature for 20 hr. Usual work-up afforded a crude product which was purified by PTLC (hexane:ethyl acetate, 85:15) to furnish **11** (6.5 mg; 45%) as a gum. IR ($CHCl_3$) : 3400, 1724, 1447, 1288, 1075, 746, 669; $^1$H NMR (80 MHz): 7.50 (d, *J*=8Hz, 2H), 7.30 (d, *J*=8Hz, 2H), 7.10 (m, H-14), 4.80 (s, H-17a), 4.75 (sbr,H-15), 4.65-4.00 (overlapping signals of H-3, H-19, H-17b); 2.50 (s,3H), 1.50 (s,H-20), 0.64 (s,H-18). No molecular ion peak was seen, other major peaks were at m/z 317 ($M^+$-$C_7H_7SO_3$), 307, 299, 286, 268, 254, 240, 224, 198, 172, 150, 97, 85, 71, 58.

**Preparation of the tosylate 12.** A solution of **6** (10mg) in dry pyridine (0.1 mL) was treated with *p*-toluenesulphonyl chloride (150 mg) and stirred at room temperature for 15 hr. The reaction was TLC monitored. Work-up afforded a crude residue which was purified by preparative TLC (hexane:ethyl acetate, 85:15) to furnish **12** (8 mg; 55%) as a gum. IR ($CHCl_3$) : 3400, 1735, 1618, 1460, 1298, 1134, 1074, 699; $^1$H NMR (80 MHz): 7.50 (d, *J*=8Hz, 2H), 7.30 (d, *J*=8Hz, 2H), 4.85 (sbr,H-17a), 4.65-4.00 (overlapping signals of H-17b, H-3, H-19, H-15); 2.50 (s,3H), 1.25 (s,H-20), 0.85 (s,H-18). No molecular ion peak was seen but other major peaks were at m/z 472 ($M^+$-$H_2O$-$C_7H_8SO_2$), 299, 284, 270, 256, 230, 200, 186, 171, 144, 118, 107, 79.

**Preparation of the iodide 13.** A solution of **12** (10mg) in dry acetonitrile (3 mL) was treated with sodium iodide (700 mg). Refluxed the reaction mixture for 36 hr and monitored by TLC. Work-up afforded a crude product which was purified by PTLC (hexane : ethyl acetate, 90:10) to furnish **13** (5 mg;

45.6%) as a gum. IR ($CHCl_3$) : 3400, 1730, 1450, 1380, 1217, 1026, 766; $^1H$ NMR (80 MHz): 4.85 (sbr,H-17a), 4.75-4.00 (overlapping signals of H-17b, H-15, H-3, and H-19); 1.25 (s,H-20), 0.75 (s,H-18). No molecular ion peak was seen, other major peaks were at m/z 318 ($M^+$-128-18), 284, 224, to 83.

**Acknowledgement**

The authors are grateful to the Director CIMAP, for providing necessary lab. facilities for this work.

**References**

1 Chaudhury P K, Singh Meenakshi, Pal Mahesh, Sharma R P & Jain S P, *Indian J Chem*, 38B, **1999,** 632.
2 Tang W, Eisenbrand G, *Chinese drugs of plant origin* (Springer Verlag, Germany), **1992**.
3 Jantan Ibrahim & Waterman P G, *Phytochemistry*, 37, **1994,** 1477.
4 Landino L M & Macdonald T L, *Chemistry and pharmacology of taxol and its derivatives*, edited by V Farin (Elsevier, New York), Chapter 7, **1995**.
5 Sharma D N & Sharma R P, *Tetrahedron Lett*, 26, **1995,** 2581.
6 Matsuda T, Kuroyanagai M, Sugiyama S, Umehara K, Ueno A & Nishi K, *Chem Pharm Bull*, 42, **1994,** 1216.