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ACTION OF CrO3/PYRIDINE COMPLEX ON URSOLIC ACID – FORMATION OF 3-OXO-11-EN-13β, 28-OLIDE AND 3, 11-DIOXO-URSOLIC ACID

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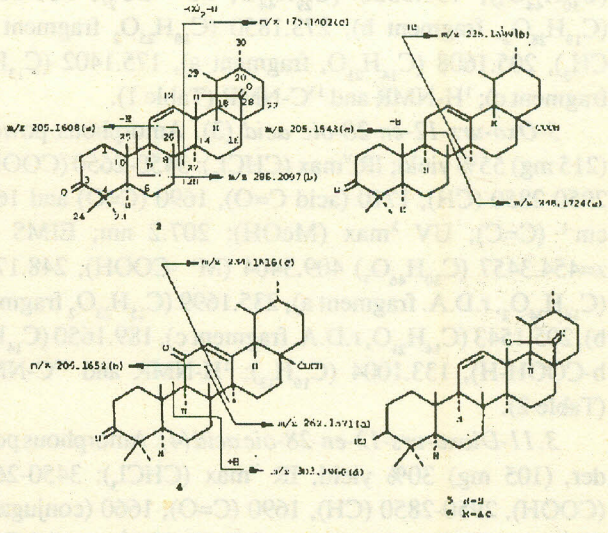
Ursolic acid on treatment with CrO_3/pyridine complex at room temperature for 3 hours furnished 3-oxo-11en-13β, 28-olide (2) and 3, 11-dioxo-ursolic acid (4) through allylic oxidation, along with the expected 3-oxo-derivative (3). This is the first report on the action of CrO_3/pyridine on ursolic acid. The allylic oxidations with this reagent are also very rare and required a prolonged treatment in previous studies.

Key words: CrO_3/pyridine complex, ursolic acid, 3-oxo-11en-13β, 28-olide, 3, 11-dioxo-ursolic acid, 3-oxo ursolic acid, allylic oxidation.

Introduction

Oxidative transformations of pentacyclic triterpenes with different oxidizing agents have attracted the attention of various groups of workers due to their potential utilization in the synthesis of natural triterpenes. In the present studies the milder oxidizing agent CrO_3/pyridine (Sarett reagent) [1] has been used for the oxidation of the pentacyclic triterpene, ursolic acid (1). The reagent is mostly used for the conversion of secondary alcohols to ketones [1], and for allylic [2] and isolated [3] primary alcohols to aldehydes and is preferred over other reagents, since it usually does not affect olefinic linkages and ether or acetal groups [2]. The present reaction was carried out at room temeperature for 3 hrs and the resulting mixture after separation through flash column chromatography furnished three products. It is interesting to note that apart from the usual oxidative product 3, two further products 2 and 4 were also obtained through allylic oxidations [4]. This reagent has rarely been used for allylic oxidation and a few instances found in literature show that the reactions had to be carried out for about 24 hrs [5] and in one case for 30 days [6]. Oxidation of ursolic acid (**1**) has been reported earlier with chromium oxide in acidic conditions (Jone's reagent) which yielded 3-keto (**3**) and 3, 11-diketo (**4**) derivatives but the lactone (**2**) was not obtained [7]. On the other hand oxidation of 1 with $KMnO_4$ resulted in the naturally occurring 11-en-13β, 28-olide [7] with the intact 3-hydroxyl function. Similarly periodate oxidation of ursolic acid and its acetate furnished the respective 11-en-13β, 28-olide only, the(-) hydroxyl and acetoxyl groups at C-3 remaining unaffected [4]. Thus the present studies provide a method for the synthesis of naturally occurring triterpenoids 3β-hydroxy-urs-11-en-13β, 28-olide (**5**) [4] and 3β-acetoxy-urs-11-en-13β, 28-olide 6 [8] with the same reagent. The formation of 5 and (**6**) can readily be envisaged through $NaBH_4$ reduction of the 3-keto derivative **2** and acetylation of the 3-hydroxy derivative respectively. Similarly, reduction of the double bond at C-11 may lead to the synthesis of another natural triterpene 3β-hydroxy-urs-13β, 28-olide [9,10].



Experimental

IR and UV spectra were measured on JASCO IRA-1 and Pye-Unicam SP-800 Spectrometers respectively. Mass spectra were recorded on Finnigan MAT-112. Exact masses of various fragments were obtained through peak matching and high resolution mass spectrum. ^1H and ^{13}C-NMR spectra were recorded in $CDCl_3$ on a Bruker AM-300 spectrometer operating at 300 MHz and 75 MHz respectively. The chemical shifts are reported in δ (ppm) and coupling constants in Hz. Assignments of ^{13}C-NMR chemical shifts are based on DEPT, hetro-COSY and comparison with similar compounds [11-13]. Flash column (model Eyela) was used with silica gel (9385) as adsorbant and hexane and hexane-ethyl acetate and ethyl acetate-methanol (gradually increasing the ratio of ethyl acetate and methanol respectively) as eluant. Purity of the products was checked on t.l.c. plates using silica gel 60 PF 254. Ursolic acid used was isolated from the leaves of Nerium

oleander [14] and identified through comparison of its data which were reported in literature [15-17].

Oxidation of ursolic acid with Sarett reagent. Chromium (VI) trioxide (1g) was gradually added in 10 ml of pyridine with constant stirring and the stirring continued for 30 mins. Ursolic acid (1) (500 mg) was dissolved in pyridine (50 ml), added into the chromium (VI) trioxide/pyridine complex and stirred for 3 hrs at room temperature. Work up of the reaction mixture at this stage in the usual manner afforded the product as a white crystallizate. This on separation through flash column chromatography furnished compounds (2-4) in the order of polarity with the solvent systems hexane-ethyl acetate (9:1;2 and 3) and ethyl acetate-methanol (9:1;4).

3-Oxo-urs-11-en-13β, 28-olide (**2**). Amorphous powder (45 mg) 15% yield; IR νmax ($CHCl_3$): 2900-2850 (CH), 1755 (lactone C=O), 1695 (C=O), 1600 (C=C) and 1145 cm^{-1} (C-O); UV λmax (MeOH): 204.2 nm; HRMS m/z=452.3292 ($C_{30}H_{44}O_3$), 408.3355 ($C_{29}H_{44}O$, M^+ -CO_2), 288.2097 ($C_{19}H_{28}O_2$, fragment b), 273.1850 ($C_{18}H_{25}O_2$ fragment b-CH_3), 205.1608 ($C_{14}H_{21}O$, fragment a), 175.1402 ($C_{13}H_{19}$, fragment c); ^{1}H-NMR and ^{13}C-NMR (Table 1).

3-Oxo-urs-12-en-28-oic acid (**3**). Amorphous powder (215 mg) 55% yield; IR νmax ($CHCl_3$): 3450-2650 (COOH), 2950-2850 (CH), 1710 (acid C=O), 1690 (C=O) and 1600 cm^{-1} (C=C); UV λmax (MeOH): 207.2 nm; EIMS m/z=454.3457 ($C_{30}H_{46}O_3$) 409.3404 (M^+ -COOH), 248.1724 ($C_{16}H_{24}O_2$, r.D.A. fragment a), 235.1699 ($C_{15}H_{23}O_2$ fragment b), 205.1543 ($C_{14}H_{21}O$, r.D.A. fragment c), 189.1650 ($C_{14}H_{21}$, b-COOH-H), 133.1004 ($C_{10}H_{13}$); ^{1}H-NMR and ^{13}C-NMR (Table 2).

3,11-Dioxo-urs-12-en-28-oic acid (4). Amorphous powder, (105 mg) 30% yield; IR νmax ($CHCl_3$): 3450-2600 (COOH), 2950-2850 (CH), 1690 (C=O), 1660 (conjugated C=O) and 1650 cm^{-1} (conjugated C=C); UV λmax (MeOH): 251.4, 202 nm; EIMS m/z = 468.3252 ($C_{30}H_{44}O_4$), 303.1966 ($C_{19}H_{27}O_3$, fragment d), 262.1571 ($C_{16}H_{22}O_3$, r.D.A. fragment a), 234.1616 (fragment a-CO, fragment c), 206.1652 ($C_{14}H_{22}O$, r.D.A. fragment b), 191.1432 (fragment b-CH_3); ^{1}H-NMR and ^{13}C-NMR (Table 3).

Results and Discussion

The molecular formula ($C_{30}H_{44}O_3$; M+, 452.3292) of **2**, the least polar compound, was obtained through high resolution mass spectrum which showed that it has 4 a.m.u. less than that of **1**. In the ^{1}H-NMR spectrum the signal of H-12 triplet was replaced by a dd at δ5.59 (J=10.30, 1.5 Hz)with the appearance of another downfield dd at δ5.57 (J = 10.30, 3.28 Hz) attributable to H-11. These observations showed that the double bond has been shifted from C12 to C-11 with the formation of a lactone ring (νmax 1755 cm-1) between C-13 and C-28. Further, the dd of H-3 disappeared due to the oxidation of 3-hydroxyl function to the keto group, which was supported by two doublets of double doublets of H-2β and H-2α at δ2.60 (J=15.84, 11.2, 7.12 Hz) and δ2.42 (J=15.84, 6.72, 3.76 Hz) respectively. The HRMS showed a significant fragment (a) at m/z 205.1608 ($C_{14}H_{21}O$) confirming the keto group at C-3 and another fragment (b) at m/z 288.2097 ($C_{19}H_{28}O_2$) supporting the lactone moiety at C-13, C-28. These data were in conformity with the structure of **2** as 3-oxo-urs-11en-13β, 28-olide.

The molecular ion peak of **3** was observed at m/z 454, the exact measurement (454.3457) of which gave the molecular formula as $C_{30}H_{46}O_3$ demonstrating that it is less of two hydrogen atoms from that of **1**. The ^{1}H-NMR spectrum showed a close similarity of **3** with **1**, except that the signals of H-3 were missing and H-2β and H-2α appeared as two doublets of double doublets at δ2.51 (J=15.84, 10.88, 7.32 Hz) and δ2.36

TABLE 1. ^{13}C AND ^{1}H-NMR ASSIGNMENTS OF **2** (HETCOR)

^{13}C	Chemical shifts	Correlated ^{1}H chemical shifts			
C	δ (ppm)	δ (ppm)	No. of H-atoms	Multiplicity	J (Hz)
1	39.13	2.1	1H-β	ddd	13.16,6.08,3.72
		1.4	1H-α	m	
2	33.85	2.60	1H-β	ddd	15.84,11.20,7.12
		2.42	1H-α	ddd	15.84,6.72,3.76
3	216.00	–	–	–	–
4	47.60	–	–	–	–
5	54.82	1.32	1H	m	–
6	19.14*	–	2H	–	–
7	30.92	1.26	2H	m	–
8	41.70	–	–	–	–
9	52.52	2.05	1H	s	–
10	36.24	–	–	–	–
11	129.47	5.57	1H	dd	10.30,3.28
12	132.77	5.95	1H	dd	10.30,1.50
13	89.32	–	–	–	–
14	42.13	–	–	–	–
15	25.67	1.73	1H	m	–
		1.25	1H	m	–
16	22.88	1.40	2H	m	–
17	45.11	–	–	–	–
18	60.75	1.70	1H	d	11.96
19	38.25	1.78	1H	m	–
20	40.35	0.87	1H	m	–
21	30.69*	–	2H	–	–
22	31.43*	–	2H	–	–
23	26.12	1.09	3H	s	–
24	20.84	1.04	3H	s	–
25	16.04	1.16	3H	s	–
26	18.58	1.09	3H	s	–
27	17.82	0.99	3H	s	–
28	178.90	–	–	–	–
29	17.24	1.05	3H	d	6.16
30	19.10	0.93	3H	d	5.92

*Values assigned from 13C-NMR (DEPT).

TABLE 2. ^{13}C AND ^1H-NMR ASSIGNMENTS OF **3** (HETCOR)

^{13}C Chemical shifts		Correlated ^1H chemical shifts			
C	δ (ppm)	δ (ppm)	No. of H-atoms	Multiplicity	J (Hz)
1.	39.30	1.88	1H-β	m	–
		1.42	1H-α	m	–
2.	34.15	2.51	1H-β	ddd	15.84,10.88,7.32
		2.36	1H-α	ddd	15.84,6.88,3.68
3.	217.85	–	–	–	–
4.	47.40	–	–	–	–
5.	55.27	1.29	1H	m	–
6.	19.56	1.44	2H	m	–
7.	32.46	1.51	1H	m	–
		1.35	1H	m	–
8.	39.50	–	–	–	–
9.	46.79	1.58	1H	dd	10.56,6.92
10.	36.71	–	–	–	–
11.	23.44	1.95	2H	m	–
12.	125.61	5.25	1H	t	3.48
13.	138.12	–	–	–	–
14.	42.07	–	–	–	–
15.	28.01	1.86	1H	m	–
		1.10	1H	m	–
16.	24.04	1.98	1H	m	–
		1.65	1H	m	–
17.	48.05	–	–	–	–
18.	52.60	2.20	1H	d	11.32
19.	39.06	1.32	1H	m	–
20.	38.84	0.98	1H	m	–
21.	30.62	1.51	2H	m	–
22.	36.71	1.68	2H	m	–
23.	26.58	1.08	3H	s	–
24.	21.17	1.01	3H	s	–
25.	15.22	1.04	3H	s	–
26.	17.01	0.81	3H	s	–
27.	23.55	1.07	3H	s	–
28.	184.29	–	–	–	–
29.	17.01	0.85	3H	d	6.44
30.	21.04	0.93	3H	d	6.16

(J=15.84, 6.88, 3.68 Hz) respectively showing the presence of 3-keto group (νmax 1690 cm^{-1}). These observations collectively led to decide the structure of **3** (-) as 3-oxo-urs-12-en-28-oic acid which was also supported by significant fragments in the mass spectrum (vide structure). An exact measurement of the molecular ion peak (468.3252) of compound **4** gave the molecular formula as $C_{30}H_{44}O_4$, demonstrating a loss of four hydrogen atoms from those of **1** and incorporation of one more oxygen atom. The salient features of ^1H-NMR spectrum were the signal of H-12 as a singlet at δ5.61 and absence of H-3 signal which was accompanied by downfield shifts of H-2α multiplet to δ2.37 and H-2β doublet of double doublets to δ2.60 (J=15.72, 10.80, 6.99 Hz) respectively. These data showed that apart from the carboxyl group, **4** has two ketonic groups at C-3 and C-11 (νmax 1690, 1660 cm^{-1}).

TABLE 3. ^{13}C AND ^1H-NMR ASSIGNMENTS OF **4** (HETCOR)

^{13}C Chemical shifts		Correlated ^1H chemical shifts			
C	δ(ppm)	δ(ppm)	No. of H-atoms	Multiplicity	J (Hz)
1.	39.81	2.92	1H-β	ddd	17.67,6.24,3.72
		1.45	1H-α	m	–
2.	34.19	2.60	1H-β	ddd	15.72,10.80,6.99
		2.37	1H-α	m	–
3.	216.93	–	–	–	–
4.	47.61	–	–	–	–
5.	55.47	1.22	1H	m	–
6.	18.77	1.45	2H	m	–
7.	32.47	not observed	2H	–	–
8.	39.12	–	–	–	–
9.	60.78	2.38	1H	s	–
10.	36.84	–	–	–	–
11.	199.18	–	–	–	–
12.	130.78	5.61	1H	s	–
13.	163.01	–	–	–	–
14.	43.91	–	–	–	–
15.	28.55	1.28	2H	m	–
16.	23.72	1.05	2H	m	–
17.	47.71	–	–	–	–
18.	52.63	2.37	1H	d	12.27
19.	38.64	1.37	1H	m	–
20.	38.64	1.05	1H	m	–
21.	30.32	1.22	2H	m	–
22.	36.09	not observed	2H	–	–
23.	26.56	1.07	3H	s	–
24.	21.39	1.01	3H	s	–
25.	15.61	1.23	3H	s	–
26.	19.05	0.94	3H	s	–
27.	20.95	0.97	3H	s	–
28.	182.56	–	–	–	–
29.	17.04	0.85	3H	d	6.39
30.	21.06	1.24	3H	d	6.69

These observations conclusively established the structure of **4** as 3, 11-dioxo-12-en-28-oic acid, also supported by mass spectral fragmentation (vide structure).

This paper describes the complete assignment of ^{13}C and ^1H-NMR chemical shifts on the basis of 2D NMR spectroscopy (COSY-45, NOESY, J-resolved and hetro-COSY experiments) which may be useful in isolation as well as synthetic studies in these type of compounds.

Compounds **2,3** and **4** were tested against various Gram positive (*bacillus subtilis, Bacillus pumilus, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus citreus, Streptococcus pyogenes,* and *Serratia marcescens*) and Gram negative (*Escherichia coli, Shigella dysenteriae, Shigella sonnei, Salmonella typhi, Salmonella paratyphi A, Salmonella schottmulleri, Klebsiella pneumoniae, Proteus vulgaris* and *Pseudomonas aeruginosa*) *bacteria* and no antibacterial effect was found at 100μg/disc concentration of these compounds.

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