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Selective reactivity of 2-mercaptoethanol with 5β,6β-epoxide in steroids from *Withania somnifera*

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ABSTRACT

2-Mercaptoethanol reacts selectively with the 5β,6β-epoxy steroids isolated from *Withania somnifera* substituting the epoxide by a six-membered oxyethylene-2′-thio ring whereas it failed to show such reactivity on 6α,7α-epoxy withasteroids. The structure of the product has been elucidated by spectroscopic methods, especially applying extensive 2D NMR methods. The anticancer activity of withaferin A was lost in the reaction product indicating that its activity is also linked to the free 5β,6β-epoxide functional group.

1. Introduction

In continuation of our interest [1–4] on the chemical studies of Indian medicinal plants, we have taken up *Withania somnifera* L. Dunal for the study of its chemo-pharmacology and genomics characterization. We have recently reported [5–8] the presence of several steroids, some of them possessing unusual chemical structures. One of the major constituents of *W. somnifera* leaves is withaferin A which has been reported to show a number of biological activities, viz., antitumour, COX-2 enzyme inhibitor, chemopreventive, antioxidant and the inhibition of human lung, colon, CNS and breast cancer cell proliferation, etc. [9–13].

Mercaptans are known for their reactivity on the multiple bonds to produce the addition products, and thioethers [14]. Epoxides, are well known to open forming the corresponding 1,2-diols [15–18]. Earlier, mercaptans have been found to interact with epoxide leading to the inactivation of the antitumour protein antibiotic, neocarzinostatin [19]. In the present study, 2-mercaptoethanol has been found to react selectively with the 5β,6β-epoxide and failed to react with the 6α,7α-epoxide of withasteroids isolated from *W. somnifera*. The anticancer activity of withaferin A disappeared when the epoxide was replaced by oxyethylene-2′-thio (1,4-oxathiane) ring indicating its active role in the biological activity.

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2. Experimental

2.1. General methods

NMR spectra were recorded on a 300 MHz Bruker AV-300 FTNMR spectrometer using TMS as internal standard. All integral values in ^{1}H NMR were 1H except where designated otherwise. FT-IR spectra were recorded on a Perkin-Elmer 1710B instruments. FAB HRMS spectra were recorded on JEOL SX 102/DA-6000 mass spectrometer using Argon/Xenon (6 KV, 100 mA) as the FAB gas.

2.2. Plant material

W. somnifera (Ashwagandha) leaves were collected from Lucknow, India in July 2001 and its identification was done by the taxonomists at CIMAP. The accession is being maintained in the institute farm. The plant genotype is deposited (No. RS-NMITLI-II.A) in the National Gene Bank at CIMAP, Lucknow for the field conservation and maintenance.

2.3. Extraction and isolation of compounds

The shade-dried leaves (850 g) were ground and defatted three times with *n*-hexane (3 × 1 l) by soaking overnight at room temperature. The spent material was further extracted with MeOH (3 × 1 l) overnight at RT. The isolation procedure has been described in our earlier publication [5].

2.4. Reaction procedure of 2-mercaptoethanol on withanolides

Withaferin A (**1a**, 50 mg, 0.106 mmol) was taken in absolute EtOH (5 ml) and to it 2-mercaptoethanol (10 mg, 0.128 mmol) was added adjusting the pH ~6.5 by HCl and kept at 37 °C for 3 h under N_2. The progress of the reaction was monitored by TLC. When **1a** was completely consumed, water (5 ml) was added and the reaction mixture was extracted with $CHCl_3$ (10 ml) three times. The $CHCl_3$ extract was washed with aqueous ammonical solution (pH ~8) and distilled water followed by drying over anhydrous Na_2SO_4. The extract was column chromatographed (1 m × 20 mm) on silica gel (50 g) by using *n*-hexane and EtOAc as mobile phase. The polarity of the mobile phase was increased by adding more EtOAc to yield **2a** (30 mg, yield 60%). Product **2a** (15 mg) was treated with acetic anhydride (1 ml) and pyridine (2 drops) overnight at room temperature. The completion of the reaction was monitored by TLC. The reaction mixture was dried completely, dissolved in water (2 ml) and extracted with $CHCl_3$ (5 ml) three times to obtain **2b** (12 mg, yield 80%). Similar reactions were repeated on other withanolides (**3**–**8**) to obtain their products as given in Table 1.

2.5. Compound 2a (5,6-De-epoxy-5β,6α-(oxyethylene-2′-thio) withaferin A)

White crystals, m.p. 232 °C. $[\alpha_D{}^{31}] = (+)15.63°$ (MeOH, $c = 0.88$). IR (KBr) $\nu = 3460$ (OH), 1710 (unsaturated δ lactone), 1690 (CH═CH—CO), 1240, 1140 (ether) and 660 (C—S) cm^{-1}. MS (HR, 70 eV): *m/z* (%) = 530.7246 $[M]^+$ calcd. for $[M(C_{30}H_{42}O_6S)] = 530.7242$. FABMS (70 eV): *m/z* (%) = 531 (15) $[M+1]^+$, 530 (15) $[M]^+$, 452 (18) $[M\text{-}C_2H_6OS]^+$, 434 (12) $[452 - H_2O]^+$, 416 (10) $[434 - H_2O]^+$, 149 (100). ^{1}H NMR (300 MHz, Py-d_5): $\delta = 6.18$, d ($J = 10.0$ Hz), H-2; 6.87, d br ($J = 10.0$ Hz), H-3; 5.31, s br, H-4; 3.45, dd ($J = 9.0$, 2.0 Hz), H-6; 2.35 and 1.85, m, H-7 and H-7′; 0.57, 3H, s, H-18; 1.57, 3H, s, H-19; 0.95, 3H, d ($J = 7.0$ Hz), H-21; 4.40, dt ($J = 13.0$, 5.5, 3.5 Hz), H-22; 2.34, 2H, d ($J = 8.0$ Hz), H-23; centered at 4.79, 2H, two d ($J = 12.0$ Hz), H-27; 2.15, 3H, s, H-28; centred at 4.11, 2H, m, —OCH_2; 3.11 and 3.03, m each, —SCH_2. ^{13}C NMR (75 MHz, Py-d_5; supported by DEPT & HSQC): $\delta = 201.7$ (C-1), 127.0 (C-2), 146.6 (C-3), 65.7 (C-4), 80.6 (C-5), 52.1 (C-6), 37.7 (C-7), 35.8 (C-8), 46.4 (C-9), 57.9 (C-10), 23.6 (C-11), 39.7 (C-12), 43.5 (C-13), 55.6 (C-14), 24.2 (C-15), 27.5 (C-16), 52.1 (C-17), 10.3 (C-18), 12.1 (C-19), 39.3 (C-20), 13.5 (C-21), 78.8 (C-22), 30.7 (C-23), 153.7 (C-24), 127.2 (C-25), 166.3 (C-26), 56.6 (C-27), 19.9 (C-28), 62.3* (—OCH_2) and 37.5 (—SCH_2).

2.5.1. Compound 2b

White crystals, m.p. 128 °C. $[\alpha_D{}^{31}] = (+)19.73°$ (MeOH, $c = 0.272$). IR (KBr) $\nu = 1710$ (unsaturated δ lactone), 1705 (OAc), 1690 (CH═CH—CO), 1240, 1140 (ether) and 660 (C—S) cm^{-1}. FABMS (70 eV): *m/z* (%) = 615 (10) $[M+1]^+$, 614 (8) $[M]^+$, 494 (24) $[M\text{-}C_4H_8O_4]^+$, 409 (30), 381 (15), 149 (100), 95 (22). ^{1}H NMR (300 MHz, Py-d_5): $\delta = 6.28$, d ($J = 10.0$ Hz), H-2; 6.61, br d ($J = 10.0$), H-3; 5.00, s br, H-4; 3.49, dd ($J = 9.0$, 2.0 Hz), H-6; 0.52, s, 3H, H-18; 1.61,

Table 1 – Yields and TLC R_f values of the 5β,6α-(oxyethylene-2′-thio) products from withanolides

Withanolide	TLC[a] R_f of reactant	Weight of reactant (mg)	TLC[a] R_f of product	Weight of product (mg)	Yield % (**2a**–**8a**)
Withaferin A (**1a**)	0.52	50	0.35	30.0	60.0
27-Deoxywithaferin A (**3**)	0.54	10	0.38	5.6	56.0
17-Hydroxy-27-deoxywithaferin A (**4**)	0.50	20	0.34	11.2	56.0
16-En-27-deoxy-withaferin A (**5**)	0.55	20	0.37	11.5	57.5
17-Hydroxy withaferin A (**6**)	0.37	20	0.28	10.0	50.0
Withanolide D (**7**)	0.48	20	0.33	9.5	47.5
14-Hydroxy withanolide D (**8**)	0.32	20	0.30	10.0	50.0
Withanone (**9**)	0.70	200	–	–	–
Withanolide A (**10**)	0.71	20	–	–	–
27-Hydroxy withanone (**11**)	0.35	20	–	–	–

[a] TLC system: $CHCl_3$:EtOAc:MeOH:C_6H_6 (70:2:4:24).

Table 2 – Some 2D NMR correlations in compounds 2a (300 MHz, Py-d_5) and 2b ($CDCl_3$)

Compound	H	δ[a]	$^1H^1H$ COSY	HMBC	NOESY
2a	S-CH_2	3.06	O-CH_2	C-6, O-CH_2	O-CH_2, H-6, H-7[b]
	O-CH_2	4.11	S-CH_2	C-5, S-CH_2	S-CH_2, H-4[b]
	H-6	3.45	H-7, H-7′	C-4, C-5, C-7, SCH_2	H-7, H-7′, S-CH_2
	H-7	2.35	H-6, H-7′,	C-5, S-CH_2, C-9, C-6[b]	H-7′, H-6, S-CH_2
	H-7′	1.85	H-6, H-7	C-6, S-CH_2	H-7, H-4, H-6
	H-4	5.31	H-3[b], H-2[b]	C-2, C-3	H-7′, H-2, H-3
	H-3	6.87	H-2, H-4	C-1, C-2, C-5	H-4, H-2
	H-2	6.18	H-3, H-4[b]	C-1[b], C-4, C-10	H-3, H-4
	H-18	0.57	–	C-12, C-13, C-14, C-17	–
	H-19	1.57	–	C-1, C-5, C-9, C-10	–
	H-21	0.95	H-20	C-17, C-20, C-22	–
	H-22	4.40	H-23, H-20	C-21, C-23, C-20	H-20′, H-21, H-23
	H-27	4.70	–	C-24, C-25, C-26	–
	H-28	2.15	–	C-23, C-24, C-25	–
2b	S-CH_2	2.90	O-CH_2		
	O-CH_2	4.17	S-CH_2		
	H-6	3.02	H-7, H-7′		
	H-7	2.05	H-6, H-7′,		
	H-7′	1.40	H-6, H-7		
	H-4	6.11	H-3[b], H-2[b]		
	H-3	6.29	H-2, H-4		
	H-2	6.01	H-3, H-4		
	H-18	0.67	–		
	H-19	1.25	–		
	H-21	0.95	H-20		
	H-22	4.40	H-23, H-20		
	H-27	4.85	–		
	H-28	2.15	–		

[a] For remaining signals see experimental.
[b] Weak correlation.

3H, s, H-19; 0.95, 3H, d (J = 7.0 Hz), H-21; 4.41, overlapping m, H-22; 2.30, 2H, d, (J = 8.0 Hz), H-23; centered at 5.17, 2H, two d (J = 9.0 Hz), H-27; 2.12, 3H, s, H-28; 4.39, 2H, m, —OCH_2; 3.00 and 2.90, m each, —SCH_2; 2.00, 3H and 2.01, 3H, s each, -OAc. ^1H NMR (300 MHz, $CDCl_3$): δ = 6.01, d (J = 10.0 Hz), H-2; 6.29, br d (J = 10.0), H-3; 6.11, s br, H-4; 3.02, dd (J = 9.0, 2.0 Hz), H-6; 0.67, 3H, s, H-18; 1.25, 3H, s, H-19; 0.95, 3H, d (J = 7.0 Hz), H-21; 4.40, dt (J = 13.0, 5.5, 3.5 Hz), H-22; 2.45, 2H, d (J = 8.0 Hz), H-23; centred at 4.85, 2H, two d (J = 9.0 Hz), H-27; 2.15, 3H, s, H-28; 4.17, 2H, dd (J = 7.0, 5.5 Hz), —OCH_2; 2.64 and 2.73, 2H, overlapping m, —SCH_2; 2.05 and 2.06, 3H each, s, —OAc. ^{13}C NMR (75 MHz, Py-d_5; supported by DEPT): δ = 200.7 (C-1), 129.0 (C-2), 142.7 (C-3), 69.3 (C-4), 80.5 (C-5), 52.4 (C-6), 36.3 (C-7), 37.8 (C-8), 46.5 (C-9), 58.9 (C-10), 24.1 (C-11), 39.9 (C-12), 43.8 (C-13), 55.7 (C-14), 24.4 (C-15), 27.7 (C-16), 52.2 (C-17), 10.4 (C-18), 13.8 (C-19), 39.6 (C-20), 12.4 (C-21), 78.8 (C-22), 30.9 (C-23), 157.8 (C-24), 122.8 (C-25), 165.5 (C-26), 59.0 (C-27), 22.0 (C-28), 64.4* (—OCH_2) and 37.8 (—SCH_2), 170.9, 20.6 and 21.0 (—$OCOCH_3$). 2D NMR (300 MHz, $CDCl_3$): see Table 2. *Supported by the values earlier reported for cyclic OCH_2 in withasteroids [20].

Table 3 – ^1H NMR data (300 MHz, Py-d_5) of compounds 3a–8a

H[a]	3a	4a	5a	6a	7a	8a
2	6.15, 1H	6.12, 1H	6.08, 1H	6.14, 1H	6.20, 1H	6.18, 1H
3	6.82, 1H	6.84, 1H	6.81, 1H	6.80, 1H	6.83, 1H	6.81, 1H
4	5.20, 1H	5.22, 1H	5.28, 1H	5.20, 1H	5.31, 1H	5.25, 1H
6	3.45, 1H	3.40, 1H	3.46, 1H	3.42, 1H	3.40, 1H	3.43, 1H
18	0.55, 3H	0.58, 3H	0.54, 3H	0.52, 3H	0.51, 3H	0.55, 3H
19	1.55, 3H	1.54, 3H	1.52, 3H	1.56, 3H	1.55, 3H	1.51, 3H
21	0.92, 3H	0.95, 3H	0.99, 3H	0.96, 3H	0.98, 3H	0.91, 3H
22	4.41, 1H	4.48, 1H	4.50, 1H	4.48, 1H	4.52, 1H	4.52, 1H
27	1.86, 3H	1.86, 3H	1.82, 3H	4.78, 2H	1.88, 3H	1.80, 3H
28	2.15, 3H	2.10, 3H	2.12, 3H	2.14, 3H	2.11, 3H	2.15, 3H
OCH_2	4.10, 2H	4.08, 2H	4.14, 2H	4.11, 2H	4.12, 2H	4.11, 2H
SCH_2	3.11,3.02, 2H	3.11,3.02, 2H	3.10,3.02, 2H	3.12,3.02, 2H	3.11,3.01, 2H	3.11,3.02, 2H

[a] Multiplicity and J value for all the compounds were similar as given in case of **2a**.

2.6. Oxyethylene-2′-thio derivatives (3a–8a) of other streroids

The products showed additional signals of —OCH_2CH_2S— in their ^{1}H NMR spectra (as in case of **2a**) in the range of (Py-d_5) δ = centered at 4.08 to 4.14, 2H, dd (J = 7.0, 5.5 Hz), for OCH_2 and 3.01 to 3.02 & 3.10 to 3.12, 2H, overlapping m, for SCH_2, besides the corresponding signals of the reactants (see Table 3).

2.7. Bioassay for anticancer activity

The anticancer activity of withaferin A (1) and its product (2a), along with several other steroids isolated from *W. somnifera*, was tested by primary screening using microculture tetrazolium assay [21] on five cell lines for ovary, pancreatic, prostate, colon and breast cancer. Serial dilutions of the test molecules were incubated for 72 h with cells attached to the walls of 96 well cultured plates. The cells were later stained with the tetrazolium dye MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] and optical densities were read at 540 nm on a plate reader. The results (mean of two experiments) are expressed as IC_{50} (compound concentration causing 50% reduction in cell viability). Interassay variation in results was <10%.

3. Results and discussion

Withaferin A (**1a**) and withanone (**9**), the two major steroids of *W. somnifera* leaves, possess several structural features in common including the 2-en-1-one, 24-ene and 22,26-lactone, besides the basic skeleton but the several biological activities are associated with **1a** while **9** lacks most of them. Recently, Jayaprakasam et al. [9], while working on the structure-activity relationships of withasteroids, have concluded that the antiproliferative activity of withanolides is mainly due to the 2-en-1-one in A ring and the hydroxyl groups at C-3, C-4 and C-27. Our study has revealed that the epoxide functional group at C-5,6 also contributes to its biological activities.

2-Mercaptoethanol has now been found to be an effective reagent to react selectively at the 5β,6β-epoxide group without influencing other functional groups of the molecule. This reactivity has enabled us to understand if the 5β,6β-epoxide also plays important role to the anticancer activity of withaferin A. Interestingly, this activity has disappeared in the reaction product (**2a**), clearly indicating that in addition to the earlier established 2-en-1-one in A ring and the hydroxyl groups at C-3, C-4 and C-27, the 5β,6β-epoxide also plays a role in the anticancer activity of withaferin A.

2-Mercaptoethanol is chemically used as reducing reagent and is generally regarded as the biochemical oxidizer, also. It is, now, being reported that this reagent reacts well with the 5β,6β-epoxide group in steroids to form a 5β,6α-oxyethylene-2′-thio (1,4-oxathiane) ring. One of the significant aspects of this reagent is that it does not react with the 6α,7α-epoxide of withanone skeleton under these conditions. It is possible that in the case of 6α,7α-epoxide, the strong interaction of the SH nucleophile with C-10 angular methyl interferes in a diaxial addition to the epoxide. The reaction was applied to several withanolides isolated from *W. somnifera*, possessing withaferin A and withanone skeletons, which supported these observations, also (Tables 1 and 3).

The structure of **2a** was elucidated by spectroscopic methods. Its ^{1}H NMR spectrum showed sets of signals similar to those in the spectrum of the reactant except the two additional signals as two multiplets at δ 3.11 and 3.03 for SCH_2 and a multiplet centered at δ 4.11 for both the protons of OCH_2. The signal corresponding to the epoxide proton at C-6 in **1a**, now, appeared as double doublet (J = 9.0, 2.0 Hz) at δ 3.45 in **2a**. These assignments, which clearly differed from those of **1a**, were confirmed by using various techniques of 2D NMR. In the ^{1}H^{1}H-COSY plot H-6 at δ 3.45 showed correlations with H-7α and H-7β at δ 2.35 and 1.85, respectively, whereas the SCH_2 at δ 3.11/3.03 and OCH_2 at δ 4.11 had a strong connectivity with each other. In the ^{13}C NMR spectrum, two additional signals appeared at δ 37.5 and 62.3 for SCH_2 and OCH_2, respectively, while the signals for C-5 and C-6 were shifted to δ 80.6 and 52.1, respectively. These interpretations were supported by the HMBC and HSQC techniques of 2D NMR spectroscopy, also (Table 2).

The epoxide ring of withaferin A, opens to form 5,6-diol when catalyzed by hydrochloric acid [14]. Consequently, the sulfur of 2-mercaptoethanol being a more powerful nucleophile than its O-analogue, might have joined at C-6 losing the —S—H proton. The preference of the attachment of S over O at C-6 could be explained by nucleophilic attack of the SH group faster than OH, followed by the SH bond cleavage and proton transfer to generate the neutral hydroxyethyl sulfide intermediate. The 5β-hydroxy-6α-thio-dimer of withaferin A, recently isolated from the same plant, also supports this type of reaction in a biological system [22]. The spectral data discussed above indicate that the linkage of SCH_2CH_2OH at C-6 acquires α-orientation after the epoxide cleavage. Thereafter, the diol at C-5 as β-OH and at C-6 as α-SCH_2CH_2OH, respectively, thus formed, may go under condensation [23] to form the product **2a** having 5β,6α- oxyethylene-2′-thio ring. The β-orientation of the 1,4-oxathiane (oxyethylene-2′-thio) ring at C-5 is based on the NOESY NMR which is the preferred probability for such substitutions [22]. In the NOESY spectrum H-4 showed correlation with H-7α, H-3 and H-2 while H-6 had correlations with H-7β, H-19 and SCH_2, clearly supporting the α-orientation of the sulfide at C-6 along with an unaltered β-oxy group at C-5 in compound **2a**. According to a Dreiding model of **2a** also, the NOESY spectrum should not show the correlation of C-5α–O–CH_2CH_2–S– with H-7α. The 0.28 ppm downfield shift of OCH_2 in the ^{1}H NMR spectrum of **2b** compared to that of **2a** might be caused by the effect of the 4-acetoxy group which further supported the β-orientation of the alkoxy group at C-5.

The stereochemistry elucidated above, was well supported by examination of a Dreiding model of the molecule. The cyclization of SCH_2CH_2OH with C-5 OH was supported by the insignificantly changed chemical shift of OCH_2 at δ 4.11 in **2a** and at δ 4.39 in **2b** excluding the possibility of a free OH available for acetylation. That the condensation occurred with C-5 OH, and not with C-4 OH, is supported by the fact that **2a** after acetylation yielded **2b** showing two acetate signals in its ^{1}H and ^{13}C NMR spectra and was quite different from **1b** (comparison with the spectra already available with us). Thus the absence of a third acetate signal in **2b** confirmed that **2a** does not have a free –S–CH_2CH_2OH which would be possible

only when these hydroxyls have undergone condensation to form an ether at C-5. Interestingly, in the ^{1}H NMR spectrum of **2b**, the standard coupling of 6.0 Hz between H-3 and H-4 was absent and also H-4 showed a stronger deshielding effect in **2a** and **2b** as compared to **1a**. These effects could be attributed to the 1,4-oxathiane ring in place of an epoxide at C-5β,6β. The structure of **2a** and **2b** were well supported by their IR and mass spectra also. An earlier paper on a molecule with an α-sulfide at C-6 forming the oxide at C-4 having an identical stereochemistry at C-6 has been reported from *Physalis peruviana* [24]. Also, the α-epoxide in steroids at C-5, C-6 has earlier been opened by thiophenol in the presence of alumina to form the C-5α-OH and C-6β-SPh supporting the inversion of stereochemistry at C-6 when reacted with sulfur reagents [25,26].

The same reaction was applied to six more withanolides isolated from *W. somnifera*, viz., 27-deoxywithaferin A (**3**), 17-hydroxy-27-deoxywithaferin A (**4**), 27-deoxy-16-en-withaferin A (**5**), 17-hydroxywithaferin A (**6**), withanolide D (**7**), 14-hydroxywithanolide D (**8**) and a new withanolide (whose structure is yet to be published) belonging to withaferin A skeleton in A/B ring. All of these withanolides gave similar products (**3a–8a**) as in case of withaferin A confirming the specific reaction of 2-mercaptoethanol on 5β,6β-epoxide withanolides. This reaction was further tried on the withanone (**9**), withanolide A (**10**) and 27-hydroxywithanone (**11**), also isolated from the same extract of *W. somnifera*. Contrarily, none of these compounds gave the same addition product, as all of them possessed the α-epoxide at C-6,7 in place of 5β,6β-epoxide. In order to find out if the reaction can further occur, we changed the conditions by raising the temperature up to 80 °C and the pH to 5.0 and 4.0. Unfortunately, a complex mixture of degraded products was obtained. In this case, it is possible that the strong interaction of the SH nucleophile with C-10 angular methyl occurs resulting in a diaxial addition to the epoxide. Therefore, from this experiment, it is con-

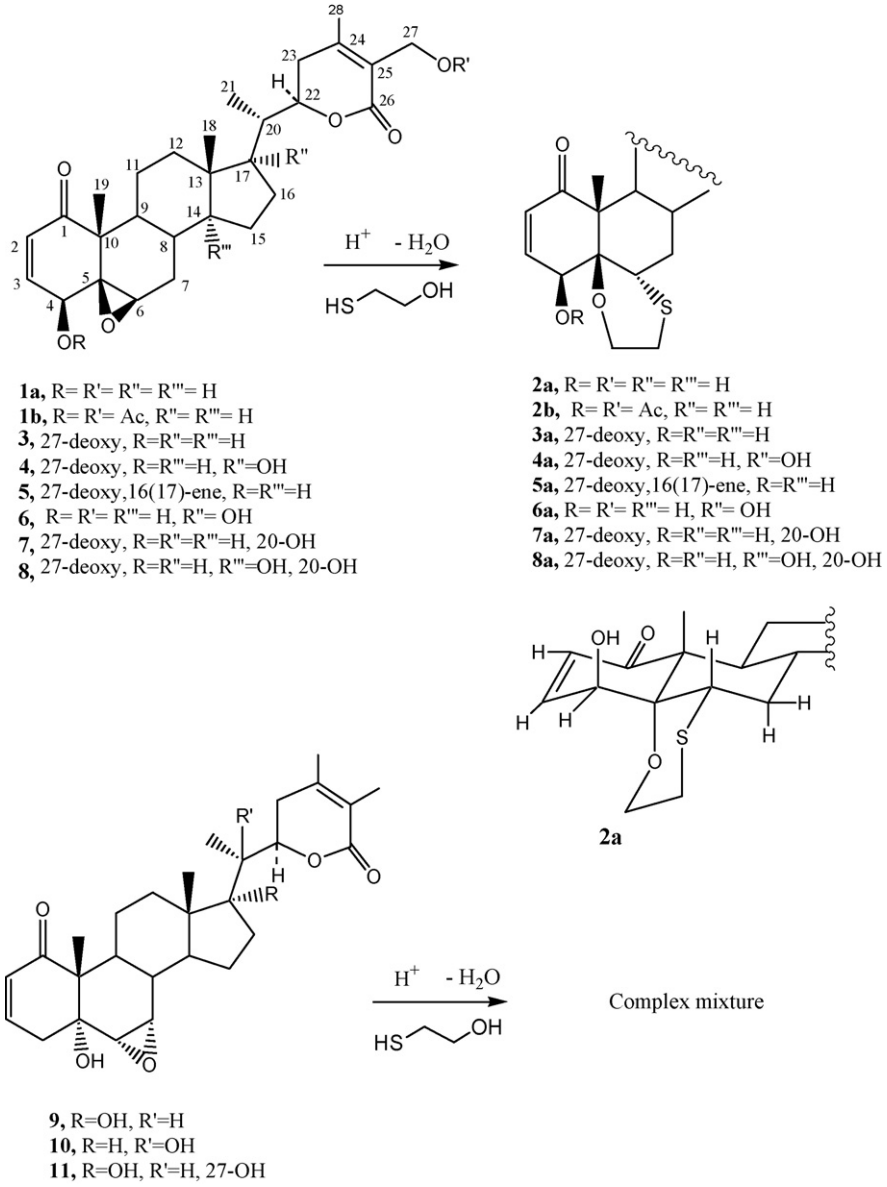

Fig. 1 – Reaction of mercaptoethanol with withaferin A (1a) to form 5,6-de-epoxy-5,6-(oxyethylene-2′-thio) withaferin A (2a).

cluded that the reaction of 2-mercaptoethanol on epoxide ring is selectively favoured at 5β,6β-position of the bridged carbon of a steroidal molecule. It is worth mentioning that the roots of *W. somnifera* contain withanolide A (**10**) (with 6α,7α-epoxide) as a major component while withaferin A (**1a**) (with 5β,6β-epoxide) was a minor constituent and the thiol-dimer of only the minor constituent could be isolated [22] which happened to be in agreement with our reaction under the laboratory conditions.

Results of the anticancer activity of withaferin A (**1**) and its product (**2a**) along with several other steroids isolated from *W. somnifera* showed that withaferin A (**1a**) exhibited activity against all the five cancer types that were tested (IC_{50}: ovary = 8.8 μg/ml, pancreatic = 8.75 μg/ml, prostate = 15.25 μ/ml, colon ≥ 20 μ/ml, breast = 19 μ/ml) while the reaction product (**2a**) lost these activities. Withanone (**9**) was also found inactive in the present screening tests. These results clearly indicated that the epoxide functionality at the 5β,6β position is necessary for the biological activities associated with the withaferin A. Similar results have earlier been reported when the loss of activity in physalolactone with same regio and stereochemistry (replacing 5β,6β-epoxide by 5βOH and 6αCl groups in withaferin A) against P-338 lymphocytic leukemia was observed [11].

4. Conclusion

The selective reaction of 2-mercaptoethanol with 5β,6β-epoxywithasteroids has shown that this reagent can be used for selective preparation of oxyethylene-2′-thio (1,4-oxathiane) ring at a bridged carbon without affecting other functional groups. This selective reaction will also be useful to ascertain the position of an epoxide either at 5,6 or 6,7 in withasteroids. *W. somnifera* is a valuable and very popular Ayurvedic (the ancient Indian system of medicine) drug associated with various therapeutic properties to match somewhat with ginseng. A number of recent studies have assessed the antistress, antiinflammatory, antitumour, antibiotic, anticonvulsant, antiageing and CNS depressant activities [11] in *W. somnifera*. Many of these activities have been attributed to Withaferin A, the major steroid found in its leaves and roots. Other steroids like, withanolides E and D show moderate cytotoxicity on melanosarcoma and leukemia. Some of the withanosides (glyco-withanolides) have been reported to show tachyphylaxis [12], COX-2 and lipid peroxidation inhibitiory activities [13]. However, among the steroids isolated in this group (Table 1), only withaferin A showed the tested anticancer activity on several cancer lines while the other compounds (**2a** and **3**–**11**) failed to exhibit such activity. The anticancer activity of withaferin A was also dependent upon the presence of 5β,6β-epoxide ring in B ring (Fig. 1).

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