THE FACILE ADDITION OF SECONDARY AMINES TO WITHAFERIN A. A FURTHER EXAMPLE OF ALUMINA-CATALYZED ADDITION OF AMINES TO α, β-UNSATURATED KETONES

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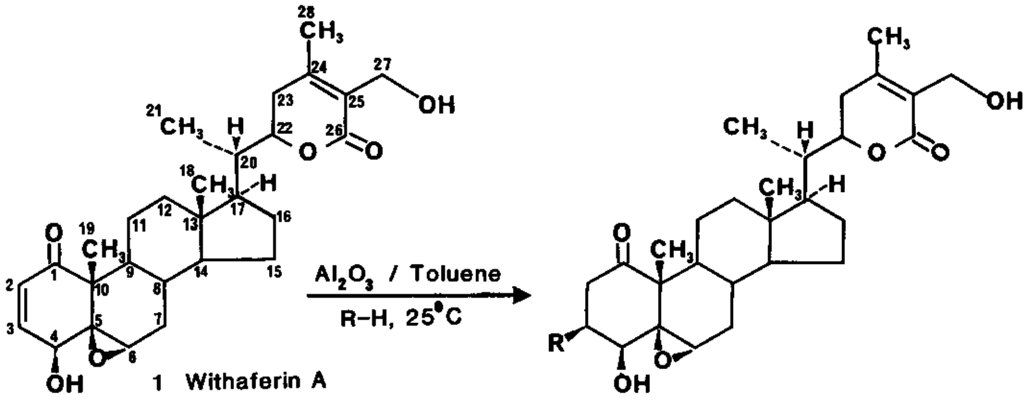
Abstract— The alumina-catalyzed addition of various secondary amines to withaferin A (1) is facile and efficient. The addition is illustrated by the preparation in excellent yield of 3-aziridinyl-2,3-dihydrowithaferin A (2), a compound with significant in vivo antitumor activity.

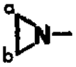
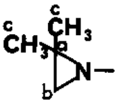
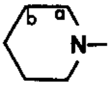
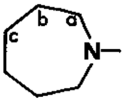
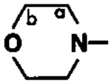
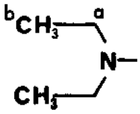
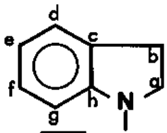
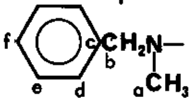
The introduction of an amine functionality in the steroid nucleus is known to alter biological activity significantly.[1] Synthetic and naturally-occurring 3-aminosteroids are known to possess important biological activities, such as CNS, anaesthetic and neuromuscular blocking.[2] For a study of the relationship of structure with antitumor activity, we required a simple and efficient method of introducing an amine functionality at the C(3) position of withaferin A (1). As earlier investigators observed[3,5] that alcohols add to the enone moiety of withaferin A with ease (especially in the presence of a base such as DBU), we anticipated that amines, and in particular secondary amines, would add in a similar fashion to give the required amino adduct. However, treatment of withaferin A (1) with aziridine or diethylamine resulted in the formation of a complex mixture of products, and the reaction did not proceed to completion within two days. Hence we took advantage of our recent discovery of the use of alumina as a catalyst for the addition of secondary amines to exocyclic α,β-unsaturated ketones.[6]

In this paper we describe an alumina-catalyzed addition of secondary amines to the enone moiety of withaferin A, a reaction which is facile and efficient. The results of addition of nine secondary amines to withaferin A are presented in Table I.



1 Withaferin A

Table I: Addition of Secondary Amines to Withaferin A

Compound	R	Reaction Time	%Yield†
2		1 hr	85
3		24 hr	65
4		1 hr	70
5		24 hr	68
6		5 hr	67
7		3 hr	86 ‡
8		24 hr	62*
9		48 hr	57*
10		24 hr	67*

†Yield is after flash chromatography.[7] All compounds were fully characterized by mass, proton & carbon-13 NMR spectra.
‡The material was triturated with $CHCl_3$/hexane before chromatography.
*Yield is based on recovered withaferin A (54, 70, and 85% conversion for compounds **8**, **9**, and **10**, respectively)

The structures of addition products were determined by ^1H and ^{13}C NMR spectral analysis. The values and assignments of the chemical signals of the ^1H and ^{13}C NMR spectra of the products are given in Tables II and III. The ^{13}C chemical shifts assignments were based on previous studies reported from our laboratory[3] and others.[8]

The following is a representative experimental procedure: 500 mg of withaferin A (**1**) was treated with 5 ml of aziridine[9] and 500 mg of Al_2O_3 (activity-III)[10] in 120 ml of toluene at 25°C. The reaction mixture was stirred under nitrogen until the starting material was consumed (TLC, 1 hr). The solid that was obtained after filtration and evaporation was subjected to flash chromatography[7] [SiO_2, 40-63 μm; 3% EtOH in EtOAc] to give 463 mg of 3-aziridinyl-2,3-dihydrowithaferin A (**2**). Crystallization from EtOAc/hexane gave compound **2** as white cubes, mp 205.5 -207.5°C (corr.).

Table II. ^{13}C Chemical Shifts and Assignments of Compounds **2-10**†

	2	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
C(1)	210.6	211.5	211.5	211.3	211.3	211.1	211.1	210.3	211.0
C(2)	39.8	36.2	38.7	36.4	37.2	36.5	37.3	36.6	37.9
C(3)	68.9	65.7	64.5	63.9	64.4	63.9	59.6	55.8	63.2
C(4)	77.1	78.0	75.8	73.3	73.2	74.0	73.8	74.3	74.0
C(5)	65.6	65.7	65.8	64.6	63.7	65.2	64.6	64.7	65.0
C(6)	61.1	60.2	61.4	59.9	59.3	61.0	60.0	59.7	60.0
C(7)	29.8	29.6	29.8	29.9	29.9	29.9	29.8	29.8	29.8
C(8)	31.1	31.1	31.2	31.1	31.2	31.4	31.2	31.3	31.3
C(9)	42.7	42.9	43.2	42.8	42.8	43.2	43.3	43.0	42.9
C(10)	50.5	50.4	50.2	49.9	49.9	50.4	49.8	50.1	50.0
C(11)	21.7	21.5	22.0	21.5	21.5	22.0	21.2	21.3	21.5
C(12)	27.3	27.2	27.2	27.3	27.3	27.3	27.2	27.2	27.2
C(13)	42.7	42.6	42.6	42.8	42.7	42.8	42.7	42.7	42.7
C(14)	56.0	56.1	55.9	56.1	56.1	56.2	56.0	56.1	56.1
C(15)	24.3	24.3	24.3	24.3	24.3	24.4	24.2	24.2	24.2
C(16)	39.2	39.2	39.3	39.2	39.2	39.3	39.1	39.1	39.1
C(17)	52.0	51.9	52.0	52.0	52.0	52.1	51.9	51.9	52.0
C(18)	11.6	11.6	11.6	11.7	11.6	11.8	11.6	11.6	11.6
C(19)	15.9	15.5	16.3	15.7	15.8	16.2	15.4	15.5	15.7
C(20)	38.8	38.8	38.7	38.9	38.8	38.9	38.8	38.8	38.8
C(21)	13.4	13.3	13.3	13.4	13.4	13.4	13.3	13.4	13.3
C(22)	78.7	78.7	78.7	78.7	78.7	78.7	78.6	78.7	78.4
C(23)	29.4	29.6	29.3	29.0	29.4	29.2	28.9	28.9	29.0
C(24)	153.1	153.3	153.3	152.9	152.9	152.9	153.2	152.9	153.2
C(25)	125.7	125.7	125.7	125.8	125.8	125.9	125.7	125.7	125.7
C(26)	167.0	167.0	166.9	167.0	167.0	167.0	166.9	166.9	167.0
C(27)	57.3	57.1	57.1	57.4	57.4	57.4	57.1	57.3	57.2
C(28)	20.1	20.0	20.0	20.0	20.0	20.0	20.0	19.9	20.0
a	26.8	65.8	51.6	50.5	51.1	50.2	42.7	48.0	37.0
b	26.4	40.9	23.3	24.5	28.9	67.2	13.1	28.3	58.4
c	-	16.9	-	26.3	26.7	-	-	129.8	138.7
d	-	-	-	-	-	-	-	124.6	127.1
e	-	-	-	-	-	-	-	118.3	128.5
f	-	-	-	-	-	-	-	127.4	128.6
g	-	-	-	-	-	-	-	107.3	-
h	-	-	-	-	-	-	-	150.2	-

† Chemical shifts are recorded in ppm downfield from TMS. The spectra were taken in $CDCl_3$ solution 15.03 MHz in the Fourier mode using a JEOL FX-60 spectrometer in conjunction with a JEC-980 computer.

Table III. The 90 MHz Proton NMR Shifts of Compounds 2-10[a]

	2	3	4	5	6	7	8	9	10
C(3)-H	2.75(m)	2.80-3.06(m)	2.87-3.05(m)	2.87(m)	2.35-2.73(m)	2.87(m)	3.10(m)	3.30(m)	2.60-2.85(m)
C(4)-H	3.41(bs)	3.33(bs)	3.52(d, J=4Hz)	3.51(d, J=5Hz)	3.10-3.50(m)	3.65(m)	3.45(d, J=5Hz)	3.56(d, J=5Hz)	3.5(bs)
C(6)-H	3.28(bs)	3.20(s)	3.20(s)	3.25(s)	b	3.25(s)	3.25(s)	3.26(s)	3.20(s)
C(18)-H_3	0.69(s)	0.68(s)	0.69(s)	0.67(s)	0.66(s)	0.69(s)	0.68(s)	0.67(s)	0.68(s)
C(19)-H_3	1.30(s)	1.25(s)	1.34(s)	1.28(s)	1.28(s)	1.35(s)	1.25(s)	1.33(s)	1.33(s)
C(21)-H_3	0.98(d, J=6Hz	0.98(d, J=6Hz	0.97(d, J=6Hz	0.98(d, J=6Hz	0.96(d, J=6Hz	0.98(d, J=6Hz)	c	0.96(d, J=6Hz)	0.97(d, J=6Hz
C(22)-H	4.48(m)	4.45(m)	4.45(m)	4.45(m)	4.40(m)	4.51(m)	4.42(m)	4.66(m)	4.40(m)
C(27)-H_2	4.38(s)	4.38(s)	4.30(s)	4.30(s)	4.30(s)	4.38(s)	4.30(s)	4.33(s)	4.25(s)
C(28)-H_3	2.05(s)	2.03(s)	2.05(s)	2.03(s)	2.03(s)	2.03(s)	2.03(s)	2.03(s)	2.02(s)
R	1.7(m)	1.09(d, J=2Hz) 1.32(d, J=2Hz 1.9(bs)	2.65-2.8(m) 1.69(m)	2.63(m) 2.44(m) 1.46(bm)	2.35-2.73(m) 1.53(bs)	3.65(bs) 2.55(bs)	2.52(q, J=7Hz 0.98(t, J=7Hz	2.83(m) 6.57-6.96(m)	2.18(s) 3.0(bs) 7.15(bs)

[a] Chemical shifts are recorded in ppm downfield from TMS. The spectra were taken in $CDCl_3$ on the Varian EM-390 NMR spectrometer.

[b] The chemical shift is part of the multiplate at δ 3.10-3.50.

[c] The chemical shift was covered by the triplet at δ 0.98.

The above results demonstrate that the alumina-catalyzed addition of secondary amines to enone systems is general and compatible with various sensitive functional groups.

A preliminary study of 3-aziridinyl-2,3-dihydrowithaferin A (2) has shown promising activity against murine P-388 lymphocytic leukemia (T/C 149 at 80 mg/kg).[11] The results of tests of compound 2 and other amino adducts against various tumor systems will be reported in due course.

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