Neurokinin Receptor Inhibitors: Fermentation, Isolation, Physico-chemical Properties, Structure and Biological Activity

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Seven neurokinin (NK) receptor inhibitors SCH 60059 (**1**), SCH 60065 (**2**), SCH 64879 (**3**), SCH 60061 (**4**), SCH 60063 (**5**), SCH 60057 (**7**), and SCH 64878 (**9**) and two uncharacterized components **6** and **8**, were isolated from the fermentation broth of a fungus taxonomically classified as an *Acremonium* sp. These compounds were isolated from the fermentation broth by ethyl acetate extraction. Purification and separation of the individual compounds were achieved by NK_1 and NK_2 assay-guided fractionation using gel filtration, reverse phase chromatography and HPLC. The NK active compounds were identified to be a family of polyhydroxy isoprenoid derivatives, including glycosylated members, by spectroscopic and degradation studies. Compounds **1**~**5** and **7** contain nine isoprene units connected in head to tail fashion and compound **9** contains fifteen isoprene units connected in a similar manner. All these compounds showed dual inhibition in NK_1 and NK_2 assays with IC_{50} values ranging from 2.5~11 μM in the NK_1 assay and 6.8~16 μM in the NK_2 assay.

Neurokinins (NK) are a family of 9 to 11 amino acid peptides with a common *C*-terminal sequence (Phe-X-Gly-Leu-Met-NH_2) which trigger a variety of inflammatory responses.[1,2] The most well characterized neurokinins namely substance P, NKA and NKB are involved in a variety of responses including pain transmission, neurogenic inflammation, bronchoconstriction and vasodilation. These peptides act principally through 7 *trans*-membrane domain G protein-coupled receptors that have been cloned and are termed NK_1, NK_2 and NK_3 and have selective affinity for substance P, NKA and NKB respectively.[3]

A number of non-peptide neurokinin antagonists have been identified and these have been useful in clarifying the pathophysiologic roles of neurokinins in a variety of diseases including asthma.[4] In particular, NK_1 selective antagonists such as CP-99994 and CP-96345 blocked several features of pulmonary inflammation namely vasodilation, microvascular leakage and tracheal mucus secretion while the NK_2 selective antagonist, SR-48698, had antibronchoconstrictive effects.[5~7] Furthermore, released neurokinins are thought to play a major role in the ongoing inflammatory response of "sensitized" sensory neurons.[8] Molecules with dual NK_1 and NK_2 antagonist activity could therefore have potentially enhanced anti-inflammatory activity.

During our search for novel neurokinin receptor inhibitors, we have isolated a series of nine novel polyhydroxy-isoprenoids from a fungal fermentation broth with dual NK_1 and NK_2 antagonist-like activity. The fungus producing these isoprenoids was identified to be an *Acremonium* sp.

In this report, we describe fermentation of the producing fungal culture, the isolation, physico-chemical properties, structure elucidation and biological activity of these novel neurokinin receptor inhibitors.

Materials and Methods

Microorganisms

The producing microorganism was isolated from a soil sample (rhizosphere of plant roots) collected in an acid mine drainage area located in the Forward Township of Pennsylvania. The fungus producing these neurokinin inhibitors was found to belong to *Acremonium* sp. based on preliminary taxonomic evaluation. The strain has been deposited in Schering culture collection with accession number SCF 1559.

Fermentation Conditions

Fermentation studies were carried out in shake flasks. Stock cultures were maintained as frozen whole broths at -80°C in a final concentration of 10% glycerol. The inoculum medium contained (g/liter) Proteus Peptone 5,

NaCl 5, KH_2PO_4 5, Yeast Extract 3, Cerelose 20, Soybean Grits 5, Antifoam 1 ml, tap H_2O to 1 liter. The pH was adjusted to 7.2 prior to autoclaving. A 250 ml Erlenmeyer flask containing 70 ml of this medium was inoculated with 2.0 ml of the stock culture. The flasks were incubated at 24°C on a rotary shaker at 250 rpm for 96 hours. Two and one half ml of this seed culture was used to inoculate another 250 ml Erlenmeyer flask containing 70 ml of the same seed medium and the flask was incubated, as above for 96 hours.

Five percent of the second germination was used to inoculate the fermentation medium containing (g/liter) Neopeptone 10, Cerelose 40, $CaCO_3$ 4, and tap H_2O to 1 liter. The pH was adjusted to 7.4 prior to autoclaving. The fermentation was carried out in a 2 liter Erlenmeyer flask containing 350 ml of the fermentation medium. The flasks were incubated at 24°C on a rotary shaker at 250 rpm for 120 hours.

Fermentation Analysis

Mycelial growth was measured as packed cell volume (PCV) by centrifuging the fermentation broth at 5000 rpm for 35 minutes. Production of the neurokinin receptor inhibitors were monitored over time by NK_1 and NK_2 assays.

Isolation

The steps leading to isolation and purification of these neurokinin receptor inhibitors are shown in Figure 1. One liter of fermentation broth was extracted with two volumes of ethyl acetate twice, the organic layer was removed from the broth, dried over anhydrous sodium sulfate and the solvent removed to yield 1.1 g of organic extract. The extract was dissolved in a minimum amount of methanol and loaded on a Sephadex LH-20 column packed in methanol. The column was eluted with methanol and the fractions were monitored by the NK_1 and NK_2 receptor binding assay. The active fractions were combined and the solvent removed to yield 0.52 g of solid. This solid was loaded on a CHP-20 column and eluted with an acetonitrile: water gradient. The fractions

Fig. 1. Isolation scheme for compounds **1**~**9**.

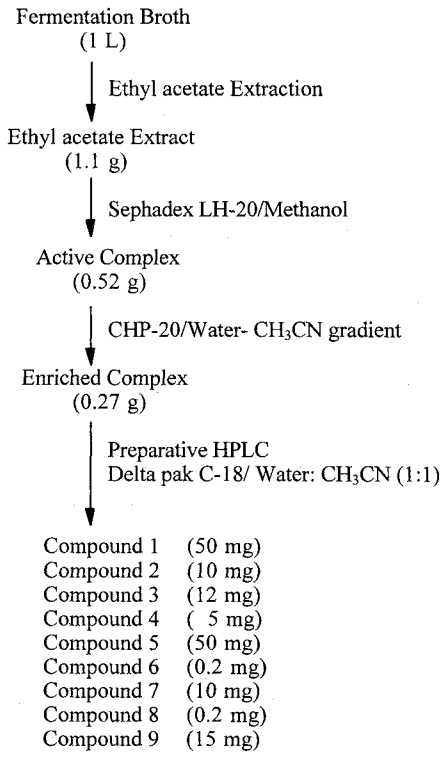
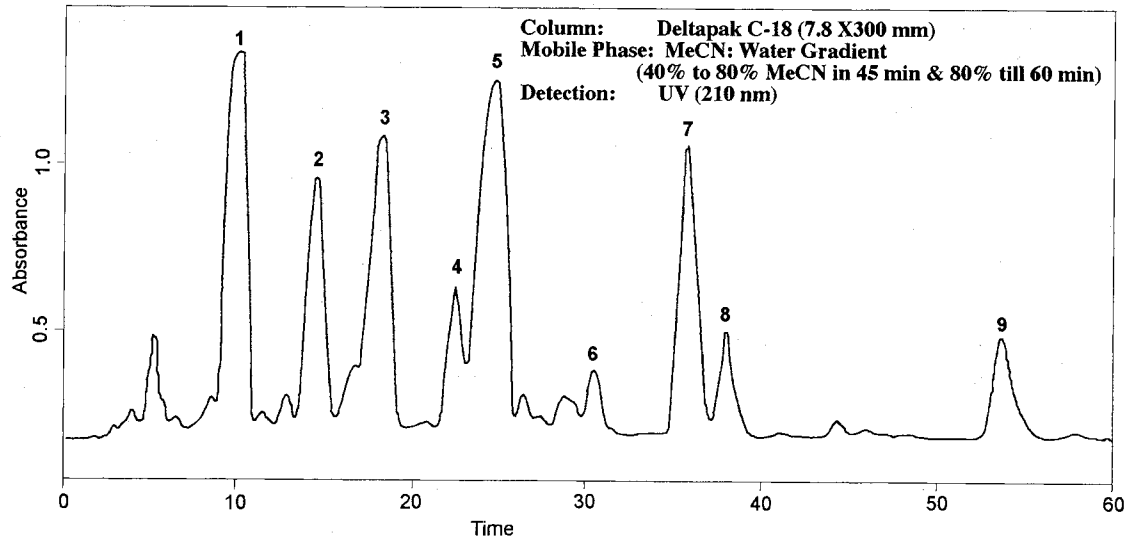


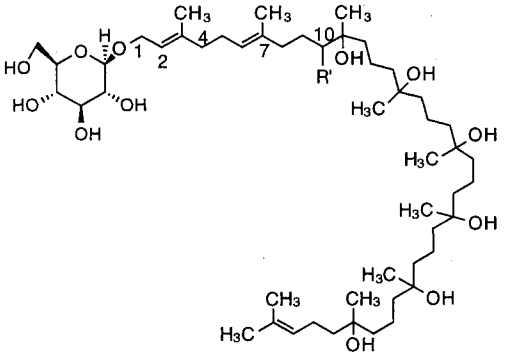
Fig. 2. HPLC profile of enriched complex.



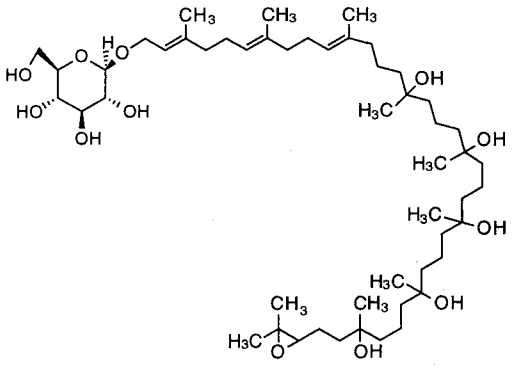
were monitored by NK_1 and NK_2 assays and the active fractions were combined, acetonitrile removed and the solution freeze dried to yield 0.27 g of enriched complex. The active compounds were separated on a preparative Deltapak C-18 column eluting with an acetonitrile: water gradient. The HPLC profile of the enriched complex is shown in Figure 2. The acetonitrile was removed from the individual peak eluates under vacuum and the remaining aqueous solutions yielded on freeze drying 50, 10, 12, 5, 50, 0.2, 10, 0.2 and 15 mgs of **1**, **2**, **3**, **4**, **5**, **6**, **7**, **8** and **9** respectively.

Physico-chemical Properties

The physico-chemical properties of the neurokinin receptor inhibitors **1**~**5**, **7** and **9** are summarized in the experimental section. Compounds **6** and **8** were charac-



1. R' = -OH
2. R' = -H



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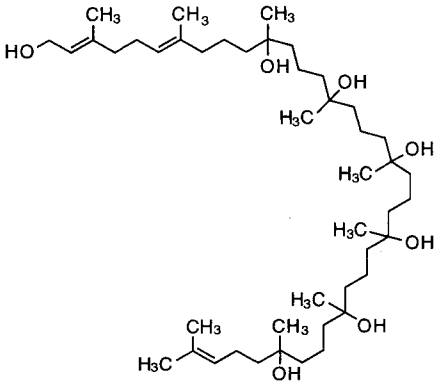
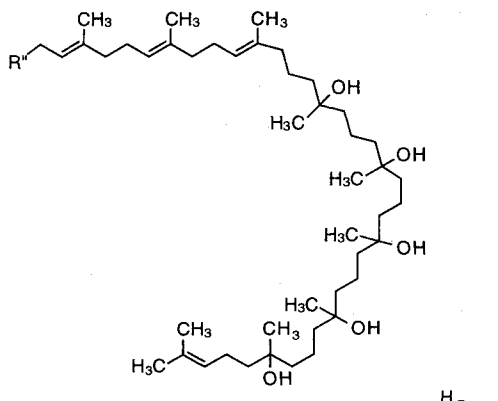
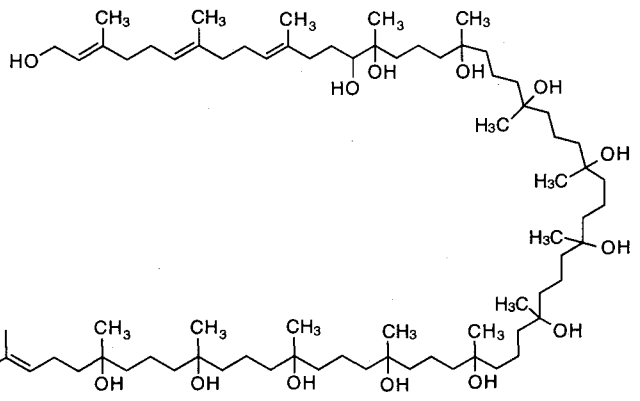


4



5. R'' = -X
7. R'' = -OH

X =



9

Table 1. Mass spectral data of **1**~**9**

Compound	Mol. ion observed in Cs^+ ion SI-MS (m/z)			Mol. wt	Mol. formula
	$(M+H)^+$	+NaCl	+KCl		
SCH 60059 (**1**)	—	939	955	916	$C_{51}H_{96}O_{13}$
SCH 60065 (**2**)	—	923	939	900	$C_{51}H_{96}O_{12}$
SCH 64879 (**3**)	—	921	—	898	$C_{51}H_{94}O_{12}$
SCH 60061 (**4**)	739	—	—	738	$C_{45}H_{87}O_7$
SCH 60063 (**5**)	—	905	921	882	$C_{51}H_{94}O_{11}$
(**6**)	1266				
SCH 60057 (**7**)	721	743	759	720	$C_{45}H_{85}O_6$
(**8**)	1308				
SCH 66878 (**9**)	1253			1252	$C_{75}H_{144}O_{13}$

terized only by mass spectrum (Table 1). All were isolated as amorphous white powders. These compounds showed only end absorption in UV spectrum. The identity of sugar residue was determined by acid hydrolysis, by stirring overnight at room temperature with 6 N HCl and subsequent TLC analysis of the hydrolysis products.[9]

Receptor Binding Assays

The human NK_1 and NK_2 receptors were expressed in CHO cells and plasma membranes were prepared as described.[10] The NK_1 receptor binding assay was performed by incubating [prolyl-3,4-^{3}H]-substance P (1 nM at 40 Ci/mmole, Amersham International) for 40 minutes at 25°C with 10 μg of CHO cell membranes expressing the human NK_1 receptor. The assay volume was 0.2 ml and the buffer (pH 7.5) contained Tris-HCl (0.05 M), $MgCl_2$ (1 mM), $MnCl_2$ (1 mM) and bovine serum albumin (1 mg/ml). The bound ligand was isolated on GFB filters (Wallac) using a Tomtek™ Mach III harvester and chilled (4°C) wash buffer containing 0.05 M Tris-HCl, pH 7.5. The filter mats were impregnated with scintillant (Meltilex™, Wallac) and bound radioactivity quantitated in a Betaplate™ (Wallac) counter. Non-specific binding was determined with a 1000 fold excess of substance P (Peninsula). The inhibitory concentration for 50% inhibition (IC_{50}) was determined graphically from dose response curves over a concentration range of 0.3 to 200 μM.

The NK_2 receptor binding assay was performed in an analgous manner to that described above with use of [4,5-^{3}H-LEU^9]-neurokinin A]-NKA (1 nM at 80 Ci/mole, Zenica) and CHO cell membranes expressing the human NK_2 receptor. Non-specific binding was determined with a 1000 fold excess of NKA (Peninsula).

Results

Production of the neurokinin inhibitors in the fermentation was monitored using ethyl acetate extracts of the aliquots obtained at different times during fermentation process. The production of neurokinin inhibitors in the fermentation peaked at 120 hours.

The ethyl acetate extract provided nine related isoprenoids. These compounds were similar in nature and were extracted into ethyl acetate and further purified and separated by using reverse phase chromatography.

Structure Determination

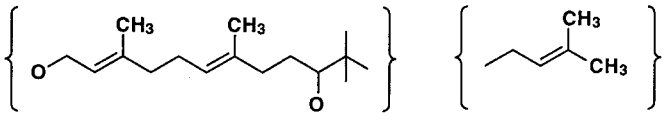
These compounds were low melting white waxy solids, soluble in methanol, chloroform, ethyl acetate but insoluble in water. Those containing sugar were more polar than others based on HPLC mobility.

SCH 60059 (**1**) a major component, in Cs^+ ion liquid secondary ionization mass spectrum (SI-MS) (Table 1), displayed an intense sodiated ion at m/z 939 $(M+Na)^+$ and on addition of potassium salt gave $(M+K)^+$ ion at m/z 955 revealing the molecular weight to be 916. Peak matching measurements using high resolution mass measurements showed the elemental composition to be $C_{51}H_{96}O_{13}$ suggesting four degrees of unsaturation. The IR spectrum displayed peaks at 3386, 2944, 1462, 1376, 1075, 755 cm^{-1} and the ^{13}C NMR spectrum measured in DMSO-d_6 at 100.5 MHz, revealed the presence of 51 carbon atoms (Table 2) and ^{13}C NMR APT experiment showed ten methyls, twenty-one *C*-methylenes, two *O*-methylenes, six sp^3 methines (>CH–O, including anomeric carbon), three sp^2 methines(=CH–), six sp^3 quaternary carbons (>C<^{O}) and three sp^2 quaternary carbons (=C<) in the molecule. The six carbon signals in the sp^2 region of the ^{13}C NMR suggested the presence of three double bonds and the methine signal at 98.61 ppm

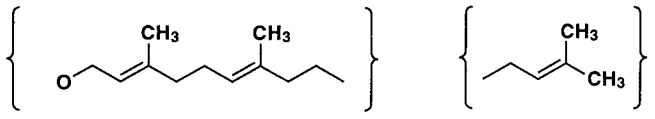
Table 2. ^{13}C NMR spectrum of **1**~**5**, **7** and **9**.

C (No.)	**1**	**2**	**3**	**4**	**5**	**7**	**9**
1	15.57 (q)	15.55 (q)	15.54 (q)	15.90 (q)	15.58 (q)	15.88 (q)	15.95 (q)
2	15.72 (q)	15.96 (q)	15.68 (q)	16.19 (q)	15.73 (q)	15.96 (q)	16.08 (q)
3	16.02 (q)	17.30 (q)	15.99 (q)	17.75 (q)	16.03 (q)	16.25 (q)	16.08 (q)
4	17.90 (t)	17.86 (t)	17.88 (t)	18.16 (t)	17.35 (q)	17.63 (q)	16.36 (q)
5	17.90 (t)	17.86 (t)	17.88 (t)	18.26 (t)	17.92 (t)	18.14 (t)	18.24 (t)
6	17.90 (t)	17.86 (t)	18.56 (t)	18.26 (t)	17.92 (t)	18.19 (t)	18.18 (t)
7	21.64 (t)	21.64 (t)	21.62 (t)	18.33 (t)	21.67 (t)	18.19 (t)	18.24 (t)
8	24.62 (q)	22.15 (t)	24.70 (q)	22.05 (t)	22.20 (t)	22.19 (t)	22.36 (t)
9	25.21 (q)	25.33 (q)	25.09 (q)	22.78 (t)	25.38 (q)	22.69 (t)	23.63 (q)
10	25.77 (t)	25.33 (q)	25.75 (t)	25.79 (q)	25.80 (t)	25.67 (q)	25.92 (t)
11	25.96 (t)	25.71 (t)	25.95 (t)	25.89 (q)	25.99 (t)	26.25 (t)	26.35 (t)
12	26.06 (q)	26.76 (q)	26.02 (q)	26.84 (q)	26.81 (q)	26.43 (t)	26.35 (t)
13	26.66 (q)	26.76 (q)	26.63 (q)	26.96 (q)	26.81 (q)	26.77 (q)	26.61 (t)
14	26.77 (q)	26.76 (q)	26.72 (q)	27.06 (q)	26.81 (q)	26.85 (q)	26.61 (t)
15	26.77 (q)	26.84 (q)	26.80 (q)	27.12 (q)	26.83 (q)	26.92 (q)	26.66 (q)
16	26.92 (q)	26.84 (q)	26.88 (q)	27.18 (q)	26.83 (q)	26.93 (q)	26.66 (q)
17	38.55 (t)	38.78 (t)	36.24 (t)	39.44 (t)	26.93 (t)	27.05 (q)	26.66 (q)
18	38.55 (t)	38.78 (t)	38.92 (t)	39.90 (t)	38.96 (t)	39.55 (t)	26.85 (q)
19	38.95 (t)	38.78 (t)	39.11 (t)	41.19 (t)	38.96 (t)	39.62 (t)	26.85 (q)
20	39.27 (t)	39.62 (t)	39.11 (t)	41.83 (t)	39.16 (t)	39.62 (t)	27.00 (q)
21	40.22 (t)	40.03 (t)	39.32 (t)	41.83 (t)	39.16 (t)	40.02 (t)	27.00 (q)
22	40.22 (t)	40.03 (t)	40.03 (t)	42.14 (t)	40.96 (t)	41.62 (t)	27.10 (q)
23	40.93 (t)	40.03 (t)	40.03 (t)	42.14 (t)	41.47 (t)	41.73 (t)	27.10 (q)
24	42.31 (t)	40.96 (t)	40.93 (t)	42.34 (t)	42.26 (t)	42.31 (t)	27.29 (q)
25	42.38 (t)	41.42 (t)	42.04 (t)	42.34 (t)	42.26 (t)	42.31 (t)	27.29 (q)
26	42.38 (t)	42.21 (t)	42.11 (t)	42.34 (t)	42.34 (t)	42.31 (t)	38.83 (t)
27	42.44 (t)	42.31 (t)	42.29 (t)	42.34 (t)	42.34 (t)	42.38 (t)	38.83 (t)
28	42.44 (t)	42.31 (t)	42.35 (t)	42.44 (t)	42.40 (t)	42.38 (t)	39.63 (t)
29	42.44 (t)	42.40 (t)	42.35 (t)	42.44 (t)	42.40 (t)	42.38 (t)	39.63 (t)
30	42.44 (t)	42.40 (t)	42.39 (t)	42.44 (t)	42.40 (t)	42.49 (t)	39.63 (t)
31	42.83 (t)	42.40 (t)	42.39 (t)	42.60 (t)	42.40 (t)	42.49 (t)	39.63 (t)
32	61.27 (t)	42.40 (t)	61.26 (t)	42.60 (t)	61.31 (t)	59.32 (t)	39.76 (t)
33	63.84 (t)	61.26 (t)	63.84 (t)	59.40 (t)	63.88 (t)	72.73 (s)	39.76 (t)
34	67.09 (d)	63.85 (t)	67.11 (d)	72.86 (s)	67.15 (d)	72.77 (s)	39.76 (t)
35	70.48 (s)	67.10 (d)	70.13 (s)	72.86 (s)	70.44 (s)	72.77 (s)	39.76 (t)
36	70.48 (s)	70.40 (d)	70.44 (s)	72.86 (s)	70.49 (s)	72.77 (s)	39.78 (t)
37	70.60 (s)	70.45 (s)	70.48 (s)	72.86 (s)	70.53 (s)	72.77 (s)	39.78 (t)
38	70.60 (s)	70.45 (s)	70.56 (s)	72.86 (s)	70.61 (s)	123.51 (d)	39.78 (t)
39	70.60 (s)	70.57 (s)	70.56 (s)	72.86 (s)	70.61 (s)	123.86 (d)	39.78 (t)
40	70.60 (d)	70.57 (s)	70.56 (d)	124.09 (d)	70.61 (d)	124.39 (d)	40.14 (t)
41	71.62 (s)	70.57 (s)	73.68 (d)	124.38 (d)	73.72 (d)	124.43 (d)	40.14 (t)
42	73.69 (d)	70.57 (s)	77.43 (d)	124.50 (d)	77.47 (d)	131.70 (s)	40.14 (t)
43	77.47 (d)	73.67 (d)	82.32 (s)	131.81 (s)	98.66 (d)	134.77 (s)	41.78 (t)
44	78.25 (d)	77.43 (d)	84.14 (d)	134.89 (s)	120.43 (d)	135.20 (s)	41.78 (t)
45	98.61 (d)	98.62 (d)	98.62 (d)	139.70 (s)	123.61 (d)	139.48 (s)	42.23 (t)
46	120.39 (d)	120.40 (d)	120.38 (s)		123.65 (d)		42.23 (t)
47	123.60 (d)	123.37 (d)	123.56 (d)		125.01 (d)		42.23 (t)
48	123.63 (d)	124.96 (d)	123.60 (d)		129.93 (s)		42.28 (t)
49	134.46 (s)	129.88 (s)	134.43 (s)		134.48 (s)		42.28 (t)
50	134.57 (s)	134.82 (s)	134.54 (s)		134.59 (s)		42.38 (t)
51	139.47 (s)	139.40 (s)	139.41 (s)		139.46 (s)		42.38 (t)
52							42.38 (t)
53							42.50 (t)
54							42.50 (t)
55							59.37 (t)
56							72.89 (s)
57							72.89 (s)
58							73.03 (s)
59							73.03 (s)
60							73.03 (s)
61							73.09 (s)
62							73.09 (s)
63							73.09 (s)
64							73.09 (s)
65							73.22 (s)
66							73.22 (s)
67							78.92 (d)
68							123.51 (d)
69							123.95 (d)
70							124.31 (d)
71							124.61 (d)
72							134.81 (s)
73							134.96 (s)
74							135.36 (s)
75							139.62 (s)

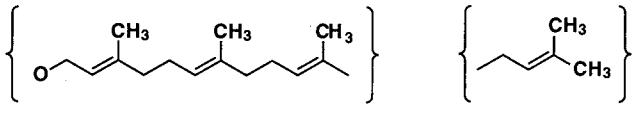
Fig. 3. Fragments derived from correlation of 2D (^{1}H-^{1}H) spectra.



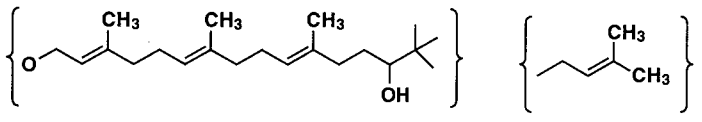
a. Compound **1**



b. Compounds **2** and **4**



c. Compounds **5** and **7**



d. Compound **9**

and other oxygenated methines suggested the presence of a sugar in the molecule. The ^{1}H NMR also showed three protons in the sp^2 region and an anomeric proton at δ 4.4, in conformity with above observations. The 2D (^{1}H-^{1}H) NMR spectrum suggested the presence of a hexose and other fragments as shown Figure 3a by adjacent proton correlation.

Acid hydrolysis of SCH 60059 followed by TLC analysis revealed the hexose to be glucose. The three double bonds and a sugar ring accounted for all the unsaturation in the molecule and suggested a linear structure for the nonsugar portion (aglycone) of the molecule. Considering the above information and similar ^{13}C chemical shifts of quaternary carbons, methylenes and methyls, we established the structure of the compound as **1**. The carbon signal at 78.25 ppm is due to the C-10 sp^3 methine in the molecule. Comparision of the coupling pattern of the anomeric proton signal and chemical shift of the anomeric carbon signal, with the literature values[11] suggested **1** to be a β-glucopyranoside. The absolute stereochemistry of the glucose was not determined.†

The mass spectrum (Table 1) of SCH 60065 (**2**) established the molecular weight to be 900 and the elemental composition to be $C_{51}H_{96}O_{12}$, suggesting a structure with one less oxygen than SCH 60059 (**1**). The ^{13}C NMR spectrum however showed only five oxygenated methines and hydrolysis confirmed the presence of glucose. The 2D (^{1}H-^{1}H) NMR spectrum revealed the presence of fragments as shown Figure 3b. Thus **2** must be a C-10 deoxygenated analog of **1**.

The molecular weight of SCH 64879 (**3**), was estab-

† Limited sample availability.

lished to be 898 by Cs^+ ion liquid secondary ionization mass spectrum (SI-MS) as shown in Table 1. High resolution mass measurements revealed the molecular formula $C_{51}H_{94}O_{12}$, two hydrogens less than **2**. ^1H NMR and ^{13}C NMR spectrum indicated the presence of only three double bonds, suggesting the presence of another ring. The ^{13}C NMR however suggested six oxygenated quaternary carbons and six oxygenated sp^3 methines. Of these one quaternary carbon appeared at 82.32 ppm and one oxygenated methine signal appeared well down field at 84.14 ppm. The oxygenated sp^3 quaternary carbon at 82.32 ppm signal was farther down field in comparision to the other five oxygenated sp^3 quaternary carbon signals near 70 ppm. Also the oxygenated sp^3 methine at 84.14 was significantly different from that of C-10 in **1**. These carbon signal shifts and consideration of degree of unsaturation in the molecule suggested that they must be linked together to form a ring. The ring was established as an epoxide using HMBC experiments and assigned the structure as **3**.

SCH 60061 (**4**) showed molecular weight 738 and the elemental composition $C_{45}H_{87}O_7$ (Table 1). The ^1H NMR and ^{13}C NMR showed the presence of three double bonds but no sugar. The composition of this compound was $C_6H_{10}O_5$ less than SCH 60065 (**2**). The 2D (^1H-^1H) NMR spectrum revealed the fragments as shown in Figure 3b. The presence of only a two proton doublet at δ 4.12 due to C-1 hydroxymethyl and absence oxygenated sp^3 methines suggested that compound **4** must be an aglycone of **2**.

The molecular weight and the elemental composition of SCH 60063 (**5**) was established using mass spectral data (Table 1) to be 882 and $C_{51}H_{94}O_{11}$ respectively. The ^1H NMR and ^{13}C NMR of **5** showed presence of four double bonds. The 2D (^1H-^1H) NMR spectrum revealed the presence of fragments as shown Figure 3c. The composition of this compound had one H_2O less than that of **2**, suggesting an additional elimination of water molecule from one of the tertiary carbon atoms. The position of this double bond was established by 2D (^1H-^1H) correlation studies as shown in **5**.

The Cs^+ ion liquid secondary ionization mass spectrum (SI-MS) of SCH 60057 (**7**) displayed an intense molecular ion at m/z 721 $(M+H)^+$, sodiated ion at m/z 743 $(M+Na)^+$ and on addition of potassium salt gave an ion at m/z 759 $(M+K)^+$ revealing the molecular weight 720. High resolution mass measurements showed the elemental composition to be $C_{45}H_{85}O_6$. The ^1H NMR and ^{13}C NMR showed the presence of four double bonds but no sugar. The 2D (^1H-^1H) NMR spectrum revealed the presence of fragments as shown Figure 3c. This compound has $C_6H_{10}O_5$ less than SCH 60063 revealing it to be an aglycone of SCH 60063 (**5**).

Table 3. Neurokinin receptor binding of **1**~**5**, **7** and **9**.

Compound	IC_{50} (μM)		
	NK_1	NK_2	NK_2/NK_1
SCH 60059 (**1**)	3	11	3.7
SCH 60065 (**2**)	7	34	4.9
SCH 64879 (**3**)	5	20	4.0
SCH 60061 (**4**)	3	14	3.7
SCH 60063 (**5**)	6	15	2.5
SCH 60057 (**7**)	6	12	2.0
SCH 66878 (**9**)	3	12	4.0

Compound **9** was significantly different compared to others. Its mass spectrum (SI-MS) displayed an intense molecular ion at m/z 1253 $(M+H)^+$, revealing the molecular weight 1252 (Table 1). High resolution mass measurements showed the elemental composition to be $C_{75}H_{144}O_{13}$. The ^{13}C NMR spectrum measured in DMSO-d_6 at 100.5 MHz, revealed the presence of 75 carbon atoms and ^{13}C NMR APT experiment showed sixteen methyls, thirty nine *C*-methylenes (of which one is *O*-methylene), one sp^3 methine (>CH–O), four sp^2 methines, (=CH–), eleven sp^3 quaternary carbons (>C<^{O}) and four sp^2 quaternary carbons (=C<) in the molecule. The eight carbon signals in the sp^2 region of the ^{13}C NMR and four protons in the sp^2 region of the ^1H NMR spectrum suggested the presence of four double bonds in the molecule. The 2D (^1H-^1H) NMR spectrum revealed the presence of fragments as shown Figure 3d. The structure was established as **9** based on these observations.

NK_1 and NK_2 receptor binding activity:

The IC_{50} values for compounds **1**~**5**, **7** and **9** in NK_1 and NK_2 binding assay are shown in Table 3. Compounds **1**, **4** and **9** showed an IC_{50} of 3 μM in the NK_1 assay and 11 μM to 14 μM in the NK_2 assay. Compound **2** was least active in both the assays with IC_{50}'s of 7 μM and 34 μM in the NK_1 and NK_2 assay respectively. The ratio of NK_2 *versus* NK_1 IC_{50} values for these compounds varied from 2 to 4.9 μM indicating that they competed for ligand binding at both receptors.

Conclusion

There is substantial evidence that activation of NK_1 and NK_2 receptors is an important step in a variety

of physiologic responses including pain transmission, smooth muscle contraction and neurogenic inflammation.[2] Appropriate neurokinin antagonists may therefore be therapeutically useful for several diseases. Several natural product structural types have been shown to inhibit NK ligand binding including the benzomalvins,[12] fiscalins,[13] anthrotainin,[14] dimerized Trp-Phe condensates,[15] and cyclic peptides.[16,17] The novel polyhydroxy isoprenoids reported here represent a new structural class of ligand binding inhibitors with dual activity on the human NK_1 and NK_2 receptors.

Experimental

General Procedures

Solvents employed for chromatography were obtained from Fisher Scientific, Fair Lawn, NJ, 07410. Sephadex LH-20 for GPC was obtained from Pharmacia LKB Piscataway, NJ 08854. The reverse phase column packing CHP-20 was obtained from Mitsubishi Kasei Corporation, Tokyo Japan. The preparative reverse phase HPLC was carried out on a C-18 silica column (Deltapak, 2.5 × 30 cm) obtained from Waters Corporation, Milford, MA 01757.

IR spectra were determined on a Nicolet FTIR model 10-MX instrument. Ultraviolet spectra were obtained by using a Hewlett Packard '8450 A' UV-vis spectrophotometer equipped with HP-9872B plotter. All Cs^+ ion liquid secondary ion mass spectra (SI-MS) and high resolution mass measurements were obtained on a VG-ZAB-SE mass spectrometer using a glycerol-thioglycerol or *m*-nitrobenzyl alcohol matrix with the sample dissolved in dimethyl sulfoxide. NMR spectra were measured on Varian instruments, XL-300 operating at 300 and 75 MHz, XL-400 operating at 400 and 100.5 MHz for ^1H and ^{13}C NMR respectively. ^1H and ^{13}C NMR, spectra were recorded relative to TMS as an internal standard. COSY spectra were measured on a Varian XL-400 instrument.

Hydrolysis of **1**

SCH 60059 (**1**) (5 mg) was dissolved 2 ml of 6 N HCl and the solution was heated at 95°C for 20 hours. The solution was diluted water and extracted with ethyl acetate. The ethyl acetate extract was dried, filtered and the filtrate was dried.

The aqueous phase on freeze drying yielded the sugar part of the molecule. It was identified as glucose by TLC analysis.

SCH 60059 (**1**): UV (MeOH) λ_{max} nm: End absorption; IR (KBr) ν_{max} cm^{-1}: 3386, 2944, 1462, 1376, 1166, 1075, 755; SI-MS: *m/z* 939 $(M+Na)^+$; HR-MS: Obsd. 939.6749, calcd. for $C_{51}H_{96}O_{13}Na$ 939.6730; ^1H NMR (DMSO-d_6) δ: 5.28 (t, J=6.5 Hz, 1H), 5.1 (dt, J=6, 0.5 Hz, 1H), 5.08 (dt, J=6, 0.5 Hz, 1H), 4.72 (b, 1H), 4.4 (s, 1H), 4.35 (b, 2H), 4.25~4.1 (m, 4H), 3.9 (b, 1H), 3.85~3.65 (m, 8H), 3.5 (m, 1H), 3.35~3.15 (b, 5H), 3.02 (m, 1H), 2.15~1.85 (m, 8H), 1.65 (s, 3H), 1.6 (m, 2H), 1.58 (s, 3H), 1.55 (s, 3H), 1.3 (b, 32H), 1.05 (s, 3H), 1.03 (s, 15H).

SCH 60065 (**2**): UV (MeOH) λ_{max} nm: End absorption; IR (KBr) ν_{max} cm^{-1}: 3383, 2945, 1463, 1376, 1074, 756; SI-MS: *m/z* 923 $(M+Na)^+$, 901 $(M+H)^+$; HR-MS: Obsd. 923.6799, calcd. for $C_{51}H_{96}O_{12}Na$ 923.6787; ^1H NMR (DMSO-d_6) δ: 5.27 (dt, J=6.5, 0.5 Hz, 1H), 5.18 (dt, J=6.5, 0.5 Hz, 2H), 4.42 (b, 1H), 4.33 (s, 1H), 4.3 (b, 1H), 4.2 (dd, J=12, 6.5 Hz, 1H), 4.1 (dd, J=12, 6.5 Hz, 1H), 3.92 (m, 1H), 3.88 (b, 6H), 3.68 (dd, J=12, 0.5 Hz, 1H), 3.59 (d, J=2 Hz, 1H), 3.45 (dd, J=12, 6.5 Hz, 1H), 3.35~3.2 (m, 8H) 2.98 (ddd, J=3, 7, 10.5 Hz, 1H), 2.1~1.85 (m, 8H), 1.62 (s, 6H), 1.55 (s, 6H), 1.28 (b, 34H), 1.1 (s, 3H), 1.08 (s, 3H), 1.0 (s, 9H).

SCH 64879 (**3**) UV (MeOH) λ_{max} nm: End absorption; IR (KBr) ν_{max} cm^{-1}: 3382, 2944, 1462, 1375, 1073, 755; SI-MS: *m/z* 921 $(M+Na)^+$, 899 $(M+H)^+$; HR-MS: Obsd. 921.6643, calcd. for $C_{51}H_{94}O_{12}Na$ 921.6643; ^1H NMR (DMSO-d_6) δ: 5.27 (t, J=6.5 Hz, 1H) 5.09 (t, J=6.5 Hz, 1H), 5.07 (t, J=6.5 Hz, 1H), 4.42 (b, 1H), 4.34 (s, 1H), 4.3 (b, 1H), 4.2 (dd, J=12, 6.5 Hz, 1H), 4.11 (dd, J=12, 8 Hz, 1H), 3.95~3.8 (b, 6H), 3.69 (dd, J=12, 1 Hz, 1H), 3.65 (t, J=8 Hz, 1H), 3.59 (d, J=3 Hz, 1H) 3.45 (dd, J=12, 6.5 Hz, 1H), 3.4~3.2 (b, 5H), 3.3 (t, J=9 Hz, 1H), 3.22 (dd, J=9, 3 Hz, 1H), 2.98 (m, 1H), 2.1~1.85 (m, 12H), 1.85~1.76 (m, 1H), 1.65 (s, 3H), 1.6 (m, 2H), 1.59 (s, 3H), 1.55 (s, 3H), 1.4~1.2 (b, 26H), 1.09 (s, 3H), 1.05 (s, 3H), 1.00 (s, 12H).

SCH 60061 (**4**): UV (MeOH) λ_{max} nm: End absorption; IR (KBr) ν_{max} cm^{-1}: 3369, 2944, 1530, 1350, 1159, 914, 732; SI-MS: *m/z* 761 $(M+Na)^+$, 739 $(M+H)^+$; HR-MS: Obsd. 939.6452, calcd. for $C_{45}H_{87}O_7$ 923.6441; ^1H NMR ($CDCl_3$) δ: 5.38 (dt, J=6.5, 0.5 Hz, 1H), 5.13 (dt, J=6.5, 0.5 Hz, 1H), 5.09 (dt, J=6.5, 0.5 Hz, 1H), 4.12 (d, J=8.0 Hz, 2H), 2.3 (b, 7H), 2.2~1.9 (m, 8H), 1.85~1.6 (b, 3H), 1.68 (s, 3H), 1.65 (s, 3H), 1.62 (s, 3H), 1.58 (s, 3H), 1.5~1.3 (b, 34H), 1.2 (s, 15H), 1.18 (s, 3H).

SCH 60063 (**5**): UV (MeOH) λ_{max} nm: End absorption; IR (KBr) ν_{max} cm^{-1}: 3369, 2944, 1712, 1461, 1384, 1075, 755; SI-MS: *m/z* 905 $(M+Na)^+$, 883 $(M+H)^+$; HR-MS: Obsd. 923.6694, calcd. for $C_{51}H_{94}O_{12}Na$ 905.6716; ^1H NMR (DMSO-d_6) δ: 5.28 (t, J=6.5 Hz, 1H), 5.19 (m,

3H), 4.7 (bs, 1H), 4.5 (b, 1H), 4.42 (m, 1H), 4.35 (s, 1H), 4.3 (d, $J=5$ Hz, 1H), 4.2 (dd, $J=12$, 8 Hz, 1H), 4.12 (dd, $J=12$, 8 Hz, 1H), 3.93 (s, 1H), 3.88 (b, 4H), 3.7 (dt, $J=12$ Hz, 1H), 3.6 (t, $J=3$ Hz, 1H), 3.45 (m, 1H), 3.25 (m, 4H), 3.00 (m, 1H), 2.1~1.85 (m, 12H), 1.63 (s, 6H), 1.56 (s, 3H), 1.54 (s, 3H), 1.52 (s, 3H), 1.4~1.4 (b, 28H), 1.02 (s, 15H).

SCH 60057 (**7**): UV (MeOH) λ_{max} nm: End absorption; IR (KBr) ν_{max} cm^{-1}: 3375, 2917, 1462, 1384, 1153, 1015, 914; SI-MS: 743 $(M+Na)^+$, 721 $(M+H)^+$; HR-MS: Obsd. 721.6346, calcd. for $C_{45}H_{85}O_6$ 721.6353; ^1H NMR ($CDCl_3$) δ: 5.4 (dt, $J=6.5$, 2 Hz, 1H), 5.1 (m, 3H), 4.15 (d, $J=7$ Hz, 2H), 2.15~1.95 (m, 12H), 1.7 (s, 3H), 1.68 (s, 3H), 1.62 (s, 3H), 1.60 (s, 3H), 1.58 (s, 3H), 1.6~1.3 (b, 38H), 1.1 (s, 12H), 1.09 (s, 3H).

SCH 64878 (**9**): UV (MeOH) λ_{max} nm: End absorption; IR (KBr) ν_{max} cm^{-1}: 3370, 2940, 1460, 1380, 1150; SI-MS: *m/z* 1275 $(M+Na)^+$, 1253 $(M+H)^+$; HR-MS: Obsd. 1275.6346, calcd. for $C_{75}H_{145}O_{13}$; ^1H NMR ($CDCl_3$) δ: 5.4 (t, $J=6.5$ Hz, 1H), 5.1 (m, 3H), 4.15 (d, $J=6.5$ Hz, 2H), 2.35~2.2 (b, 13H) 2.15~1.9 (m, 12H), 1.75 (m, 1H), 1.65 (s, 3H), 1.6 (s, 9H), 1.58 (s, 3H) 1.5~1.3 (b, 64H), 1.22 (s, 3H), 1.2 (s, 3H), 1.18 (s, 3H), 1.6 (s, 21H) 1.5 (s, 3H).

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