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Chemical Investigation of *Mycale mytilorum* and a Study on Toxicity and Antidiabetic Activity of 5-Octadecylpyrrole-2-carboxaldehyde

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Abstract—Chemical investigation of *Mycale mytilorum* afforded four new and two known compounds, of which 5-octadecylpyrrole-2-carboxaldehyde (**1**) and (6′*Z*)-5-(6′-heneicosenyl) pyrrole-2-carboxaldehyde (**2**, congeners of alkylpyrrole carboxadehyde), (2*S*,3*R*,4*E*)-1,3-dihydroxy-2-[(heneicosanoyl) amino]-4-heneicosene (**5**, sphingolipid) and 2-methyl-3-(4,5,7-trihydroxy-8-hydroxymethyltetrahydro-6H-4-pyranyl)-2-propenoic acid (**6**, tetrahydropyran derivative) are new, and batylalcohol (**3**) and *p*-hydroxyphenylacetic acid (**4**) are known. The toxicity and antidiabetic activity of 5-octadecylpyrrole-2-carboxaldehyde were evaluated for the first time. Also, compounds **1, 2, 5** and **6** were studied for the antibacterial, antifungal and antiviral activity.

Introduction

Marine organisms are known to be a prolific source of secondary metabolites which possess interesting biological activities.[1,2] In a hypothetical study by co-group, the ethyl acetate extract of *Mycale mytilorum* showed 27% reduction in blood glucose level of normal rats at a dose of 250 mg/kg body wt. Also, a large number of pyrrole analogues were reported[3–6] to exhibit diverse pharmacological activities. Recently Ortega et al. reported[7] a series of new cytotoxic metabolites, 5-acyl-2-hydroxymethylpyrroles named as mycalazols together with two 5-alkylpyrrole-2-carboxaldehydes, mycalazals 1 and 2 from the sponge *Mycale micracanthoxea*. In light of these facts, as a part of ongoing work[8] on the chemical and pharmacological investigation of marine organisms, we have taken up the chemical examination of the sponge *M. mytilorum* with special reference to the toxicity and antidiabetic activity of 5-octadecylpyrrole-2-carboxaldehyde which was isolated in reasonably good quantity.

Keywords: Mycale mytilorum; 5-octadecylpyrrole-2-carboxaldehyde; toxicity; antidiabetic studies.
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Results and Discussion

Chemistry

Compound 1. The IR spectral bands at 3290 (N–H group), 1640 (carbonyl), 3040 (C–H*str*), 1610 and 1460 (unsaturation), 1395 (aldehydic C–H*bend*), 2910 and 2820 cm^{-1} (long chain methylenes) and UV absorption maxima at 302 nm (ϵ 16,000) suggested the presence of typical pyrrole-2-carboxaldehyde.[7,9–12] ^{13}C NMR DEPT signals (see Table 1 for NMR data) at δ 178.03 (d, aldehyde), 144.09 (s), 131.80 (s), 123.17 (d) and 109.40 (d) and 31.88–27.80 (t, several methylenes) indicated that **1** is a typical 5-alkylpyrrole-2-carboxaldehyde.[7,9–14] Also, an aldehyde signal at δ 9.34 (s, 1H), two pyrrole proton signals at δ 6.90 (m, 1H) and 6.07 (m, 1H) both of which are coupled to a broad NH signal at δ 10.47 (br s, 1H) and the long chain methylene signals at δ 2.67 (t, 2H, $J=7$ Hz), 1.65 (m, 2H), 1.25 (br s, 30H) and 0.88 (t, 3H, $J=6.8$ Hz) in the ^1H NMR spectrum indicated the presence of above skeleton. D_2O exchange of the NH proton, in **1**, converted the signals of pyrrole protons to sharp doublet each with a coupling constant of 3.6 Hz, a typical value for $J_{3,4}$ in pyrroles[11–13] established the position of aldehyde and alkyl chain on C-2 and C-5, respectively. This was further supported by ^1H–^1H COSY spectra which showed a coupling between two pyrrole protons 3-H and 4-H at δ 6.90 and 6.07, respectively. The EIMS of **1** showed

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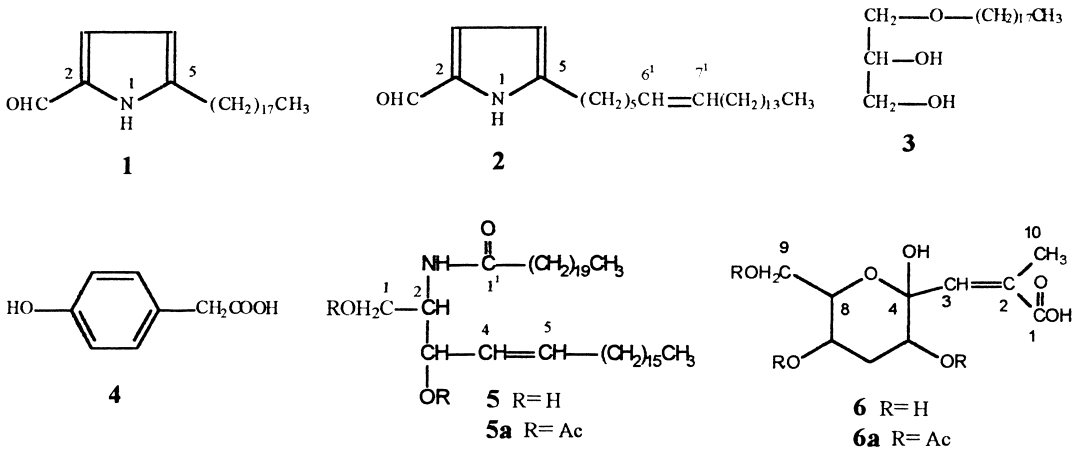


peaks at m/z 347 (M^+), 108 ($M^+-(CH_2)_{16}CH_3$), 80 ($C_5H_6N^+$), 332, 318, and 304 followed by the loss of several homologous sequence ions 290, 276 and 262 suggested octadecanyl side chain on the pyrrole moiety.[11,13] From the above spectral data, **1** was established as 5-octadecylpyrrole-2-carboxaldehyde, an another homologue to the earlier series reported.[7,11,14]

Compound 2. The close proximity in IR, UV, ^{1}H NMR and ^{13}C NMR DEPT spectral data of **2** to that of **1** and the presence of two additional olefinic carbons, δ 129.7 and 128.3 and protons, δ 5.34 (t, 2H, $J=5.0$ Hz) along with aliphatic chain signals in the former indicated the presence of double bond in the long aliphatic chain. The two olefinic protons of **2** appeared at δ 5.34 as triplet with a small coupling constant, $J=5.0$ Hz and its chemical shift value is close to that of 5-(6′-dodecenyl or tricosenyl) pyrrole-2-carboxaldehydes isolated from the sponge *Laxosuberites* sp. (lit.[11] δ 5.34), suggested the '*Z*' geometry for the alkene bond. The strong IR absorption band at 720 cm^{-1} and absence of signals of vinylic methylene carbons in the range of 33–35 ppm, also indicated that the olefinic bond has the '*Z*' geometry.[11] The signals appeared at δ 2.22 (m, 2H) and 2.01 (m, 2H) were assigned to vinylic methylene groups of the alkyl side chain.[11] The EIMS of **2** gave molecular ion peak at m/z 387 (M^+). The prominent peak at m/z 108 and the base peak at m/z 80 corresponding to the ions '**A**' and '**B**' respectively, indicated the loss of eicosenyl moiety from the molecular ion.

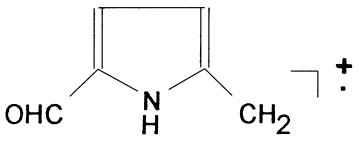
The appearance of the homologous sequence ions at m/z 122, 136, 150, 164 and 178 following the fragment ion at m/z 108 and the sequence interruption of the homologous ions at m/z 193 with the resumption of the normal homologous sequence ions (207; 221; 235; 249; 263; 277; 291, 292; 306; 320) indicated that the olefinic bond of the alkyl chain is separated from the pyrrole ring carbon by five methylene groups (cleavage of C-6′, C-7′ double bond). On comparison of the concised spectral data of **2** with those congeners, 5-(6′-dodecenyl) or 5-(6′-tricosenyl) pyrrole-2-carboxaldehydes and 5-alkylpyrrole-2-carboxaldehydes,[7,11,13] **2** was characterised as (6′*Z*)-5-(6′-heneicosenyl) pyrrole-2-carboxaldehyde.

Compound 5. The IR spectrum exhibited absorption bands of hydroxyl and amide NH (3500–3100 cm^{-1}), amide carbonyl (1640 cm^{-1}), unsaturation (1610, 1470 cm^{-1}) and long aliphatic chain (2910–2840 cm^{-1}). The diacetyl derivative, **5a** obtained from **5** with pyridine-Ac_2O, showed still sharp IR absorption band at 3340 cm^{-1} indicating the presence of amide NH. ^{13}C NMR DEPT spectrum of **5** (see Table 2 for NMR data) showed the signals at δ 173.60 of a carbonyl carbon and δ 56.93 of a methine carbon, both connected to amide nitrogen, indicating that **5** was a sphingolipid.[15–18] In

Table 1. ^{1}H NMR, ^{13}C NMR DEPT and ^{1}H–^{1}H COSY spectral data of **1**

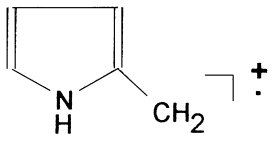
H or C no.	^{1}H NMR (300 MHz, $CDCl_3$) δ (multiplicity, proton integration, J in Hz)	^{13}C NMR DEPT (75 MHz, $CDCl_3$) δ (multiplicity)	^{1}H–^{1}H COSY correlation (300 MHz, $CDCl_3$)
1 (NH)	10.47 (brs, 1H)	—	—
2	—	131.80 (s)	—
3	6.90 (m, 1H)[a]	123.17 (d)	3-H, 4-H
4	6.07 (m, 1H)[a]	109.40 (d)	—
5	—	144.09 (s)	—
6	2.67 (t, 2H, $J=7$)	31.88 (t)	6-H_2, 7-H_2
7	1.65 (m, 2H)	29.91 (t)	7-H_2, 8-H_2
8...21	1.25 (br s, 28H)	29.63–27.80 (t)	8-H_2...22-H_2
22	1.25 (br s, 2H)	22.64 (t)	22-H_2, 23-H_3
23	0.88 (t, 3H, $J=6.8$)	14.06 (q)	—
CHO	9.34 (s, 1H)	178.03 (d)	—

[a]These signals look like triplet each ($J=3.1$ Hz) on the δ scale expansion spectra of the 90 MHz instrument. Actually, in 5-alkypyrrole-2-carboxaldehydes, these signals were reported as multiplets by Stierle et al.,[11] triplet each by Cimino et al.[13] and doublet–doublet each ($J=3.5$ and 2.4 Hz) by Ortega et al.[7]



m/z 108[M$^+$ – $(CH_2)_4CH{=}CH(CH_2)_{13}CH_3$]

A



m/z 80[MH$^+$ – $(CH_2)_4CH{=}CH(CH_2)_{13}CH_3$,CHO]

B

addition to above, the spectrum exhibited the signals at δ 62.25 (t) and 73.40 (d) corresponding to -CH_2OH and -CHOH, respectively. The identity of **5** as sphingolipid was also confirmed from the ^{1}H NMR spectrum by the presence of a characteristic[15–18] amide NH doublet at δ 6.35 (d, 1H, J = 8 Hz, exchangeable with D_2O) and methine proton connected to amide NH as multiplet at δ 3.90 which is coupled with an oxygenated methine proton at δ 4.28 (br s, 1H) and methylene protons at δ 3.69 (d, 1H, J = 10 Hz) and 3.33 (br s, 1H). The carbon and proton resonances at δ 132.35 (d), 135.87 (d) and δ 5.53 (dd, 1H, J = 15, 6.2 Hz) and 5.77 (m, 1H), respectively, indicated the presence of a double bond in the molecule. Multiplicity of one of the olefinic proton as doublet–doublet indicated that this proton is connected to an oxygenated methine proton at δ 4.28 of the sphingosine base. The presence of a multiplet at δ 5.77 of the olefinic proton indicated that this proton is coupled with the other olefinic proton (δ 5.53) and methylene protons of the long fatty alkyl chain of the sphingosine base. Thus the double bond is placed at C-4 (olefinic bond at C-4 commonly observed in several sphingolipids[15–19]). The characteristic[18,20] signals at δ 36.96 (t) in the ^{13}C NMR spectrum and δ 2.22 (t, 2H, J = 6.6 Hz) in the ^{1}H NMR spectrum suggested that the methylene function of the fatty acyl chain is attached to amide carbonyl only. Both the carbon and proton spectra exhibited also the signals of methylene and terminal methyl groups of the fatty acyl/alkyl chains that are present in the sphingolipids.

Further the position of the double bond on the alkyl chain of the sphingosine base and the alkyl chain length of the base and acyl moieties were established by means of FABMS and EIMS fragmentation pattern based on the earlier literature reports.[15,16,18,20] The FABMS of **5** showed molecular ion at m/z 649 (M^+, $C_{42}H_{83}NO_3$) in addition to the fragment ions m/z 647 (M^+-2H) and 631 (M^+-H_2O). The disappearance of the fragment ions from the ion m/z 591 followed by the next fragment ions at m/z 368 ($M^+-(CH_2)_{19}CH_3$; $(649–281)^+$) and 340 ($M^+-CO(CH_2)_{19}CH_3$; $(649–309)^+$) formed by the elimination of a fatty acyl group from the base moiety, suggested that the molecular weight of the fatty acid is 310 and that the base is sphingosine. The EIMS of **5** gave the highest mass peak at m/z 368 along with the ion at m/z 340 without a molecular ion peak indicated the loss of the fatty acyl group from the base. Indeed, highest peak at m/z 368, corresponding to the sphingosine base, observed from the EIMS of acid hydrolysed (10% HCl in MeOH) product of **5**. The EIMS and FABMS of **5** showed an interruption at the ion m/z 238 with the loss of homologous sequence ions m/z 225, 211, 197, 183, 169 indicating the cleavage of C-4–C-5 double bond of the alkyl chain of the sphingosine base by the loss of hexadecanyl moiety ($(CH_2)_{15}CH_3$).

Table 2. ^{1}H NMR, ^{13}C NMR DEPT and ^{1}H–^{1}H COSY spectral data of **5**[a]

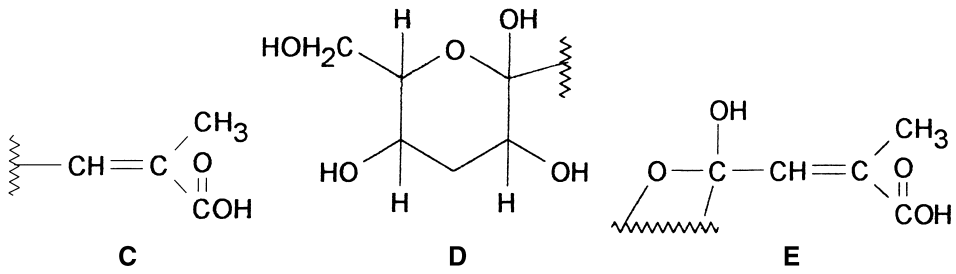
H or C no.	δ ^{1}H, ppm (400 MHz, $CDCl_3$) (multiplicity, proton intergration, J in Hz)	δ ^{13}C DEPT, ppm (50 MHz, C_5D_5N)	^{1}H–^{1}H COSY correlation[b] (200 MHz, $CDCl_3$)
1_a	3.69 (d, 1H, J = 10)	62.25 (t)	1_a-H, 1_b-H; 1_a-H, 2-H; 1_b-H, 2-H
1_b	3.33 (br s, 1H)	—	—
2	3.90 (m, 1H)	56.93 (d)	2-H, 3-H; 2-H, NH
3	4.28 (br s, 1H)	73.40 (d)	3-H, 4-H
4	5.53 (dd, 1H, J = 15, 6.2)	132.35 (d)	4-H, 5-H
5	5.77 (m, 1H)	135.87 (d)	5-H, 6-H_2
6	2.05 (m, 2H)	32.79 (t)	6-H_2, 7-H_2
7–19	1.25 (br s, 26H)	30.31 (t)–26.49 (t)	7-H_2...20-H_2
20	1.25 (br s, 2H)	22.99 (t)	20-H_2, 21-H_3
21	0.87 (t, 3H, J = 6.6)	14.32 (q)	—
1′	—	173.60 (s)	—
2′	2.22 (t, 2H, J = 6.6)	36.96 (t)	2′-H_2, 3′-H_2
3′	1.62 (m, 2H)	32.19 (t)	3′-H_2, 4′-H_2
4′–19′	1.25 (br s, 32H)	30.31 (t)–26.49 (t)	4′-H_2...20′-H_2
20′	1.25 (br s, 2H)	22.99 (t)	20′-H_2, 21′-H_3
21′	0.87 (t, 3H, J = 6.6)	14.32 (q)	—
NH	6.35 (d, 1H, J = 8)	—	2H, NH

[a]Signals of the nearly identical values may be interchangable. Signals assigned to vinyl carbons may be exchanged. Assignments made are based on DEPT, ^{1}H–^{1}H COSY spectral data.
[b]Type of spin interaction observed is vicinal coupling among all the protons and the coupling between 1_a-H, 1_b-H is geminal coupling.

The ^{1}H NMR assignments made were confirmed by the analysis of ^{1}H–^{1}H COSY spectral data. The relative stereochemistry of **5** was predicted to be the same as that of natural *erythro*-docosasphinga-4,8-dienine isolated from *Anemonia sulcata*[18] and synthetic *N*-octadecanoyl-D-*erythro*-sphingosine.[15,21] The stereochemistry was determined on the basis of ^{13}C NMR and ^{1}H NMR chemical shift values and by the comparison, since the chemical shifts ($CDCl_3$) of C-2 (δ 54.77) and C-3 (δ 74.35) of **5** are in agreement with those of *erythro*-docosasphinga-4,8-dienine (δ 54.93 (C<u>H</u>–NH) and 74.55 (C<u>H</u>–OH), respectively) and *N*-octadecanoyl-D-*erythro*-sphingosine (δ 54.7 (C<u>H</u>–NH) and 73.1 (C<u>H</u>–OH), respectively). Further, the ^{1}H NMR chemical shifts ($CDCl_3$) of 2-H (δ 3.90) and 3-H (δ 4.28) of **5** are well in agreement with those of *erythro*-docosasphinga-4,8-dienine (δ 3.9 (C<u>H</u>–NH) and 4.29 (C<u>H</u>–OH), respectively). The coupling constants of $J_{1,2}$, $J_{2,NH}$ and $J_{2,3}$ for the diacetyl derivative of **5** (6.0 and 3.9, 9.0, 6.4 Hz, respectively) are very close to those of *N*,*O*,*O*-triacetyl-D-*erythro*-sphingosine (6.0 and 3.9, 9.3, 6.0 Hz, respectively[15]). Moreover, the specific rotation of **5** −11.0° ($c=0.1$, 1-PrOH) and its diacetyl derivative, −13.5° ($c=0.1$, 1-PrOH) is very close to that of the (2*S*,3*R*,4*E*,8*E*,10*E*)-2-acetamido-1,3-diacetoxy-9-methyl-4,8,10-octadecatriene, −12.5° and *N*,*O*,*O*-triacetyl-D-e*rythro*-sphingosine, −11.8°.[15] The 4,5-alkene bond of **5** was found to be *E*, as evidenced by the large coupling constant, 15.0 Hz (15.3 Hz for ophidiacerebroside C and 15.5 Hz for *erythro*-docosasphinga-4,8-dienine[15,18]). From the above data, absolute configuration of **5** was found to be 2*S*, 3*R* and 4*E*. Based on the foregoing ^{1}H NMR, ^{13}C NMR, mass spectral and polarographic data, **5** was characterised as (2*S*,3*R*,4*E*)-1,3-dihydroxy-2-[(heneicosanoyl) amino]-4-heneicosene.

Compound 6. The IR spectrum showed very broad hydroxyl band at 3300–3600 cm^{-1} and broad O–H*str* absorption of carboxylic acids centred around 3000 cm^{-1}. The carbonyl function with α,β-unsaturation appeared at 1690 cm^{-1} and its conjugation bands appeared at 1650 and 1430 cm^{-1}. UV spectrum showed absorption maxima at 219 nm (ϵ 9500) in ethanol indicating the presence of unsaturation in the alkyl chain. Triacetyl derivative (Ac_2O:C_5H_5N) of **6**, still showed an infrared hydroxyl absorption band at 3500–3350 cm^{-1} indicating the presence of tertiary or hindered hydroxyl group. The ^{13}C NMR DEPT spectrum of **6** (see Table 3 for NMR data) showed 10 carbons corresponding to methyl (δ 12.02), methylenes (δ 62.49 and 41.45), methines (δ 136.07, 88.94, 85.56 and 71.62) and quaternary carbons (δ 165.13, 152.03 and 110.61). The quaternary carbon at δ 165.13 was assigned to the α,β-unsaturated acid carbonyl carbon. The down field signal attributed to α-carbon of the α,β-unsaturated acid system at δ 152.03 was perhaps the presence of methyl substitution and the methyl carbon on the double bond appeared as high field signal at δ 12.02. The olefinic β-carbon appeared at δ 136.07. From the above data, part structure **C** of α,β-unsaturated acid was deduced. The quaternary carbon, δ 110.61, bearing the hydroxyl group was assigned to a carbon substituted with the α,β-unsaturated acid chain. The spectrum also showed resonances of two methine carbons with hydroxyl groups at δ 85.56, 71.62 and the other oxygenated methine carbon (α-carbon of the pyran ring) at δ 88.94. Lower field signals of the oxygenated carbons insinuated the presence of a cyclic skeleton. Further, the ^{13}C NMR signal of the oxygenated methine carbon at δ 88.94 indicated that this methine carbon might be connected to the oxygen atom present in the cyclic system, i.e. tetrahydropyran ring. A hydroxyl bearing methylene carbon which appeared at δ 62.49 could be assigned as a α-substituent of the tetrahydropyran ring. The above value almost corroborates with the C-6 hydroxy methylene (lit.[22] at δ 62) of the glucopyranose moiety. A signal at δ 41.45 was assigned to the nonoxygenated methylene carbon of the tetrahydropyran moiety. Thus from the data and partial data comparison with the similar skeleton,[22] tetrahydropyran moiety **D**, was deduced. It is also evident from the data that the tertiary hydroxyl bearing carbon (δ 110.61, s) of the tetrahydropyran moiety is only the possible position for the attachment of α,β-unsaturated acid side chain. The ^{1}H NMR spectrum of **6** showed broad singlet of an acid proton at δ 13.06.[23] The high frequency signal located at δ 8.11 (s, 1H) is due to the β-proton of the α,β-unsaturated acid. The singlet appeared at δ 1.87 due to the presence of a tertiary methyl group located on the double bond. From the above data, thus the extended structure **E**, of α,β-unsaturated acid side chain was deduced.

^{1}H NMR spectrum also showed three oxygen bearing protons, of high frequency, at δ 6.97 (t, 1H, $J=6.8$ Hz), 5.02 (br s, 1H) and 4.46 (br s, 1H), probably the methine protons of the pyran ring system. The signal appeared at δ 2.65 (m, 2H) was assigned to the methylene protons (6-H_2), of the pyran moiety, connected to two hydroxy methine carbons. The hydroxyl bearing methylene protons are located at δ 4.18 (dt, 2H, $J=8$, 16 Hz). The ^{1}H NMR spectral assignments are further confirmed by ^{1}H–^{1}H COSY spectral data. The 2D-COSY spectrum



C D E

Table 3. ^{1}H NMR, ^{13}C NMR DEPT and ^{1}H–^{1}H COSY spectral data of **6**[a]

H or C no.	^{1}H NMR (400 MHz, C_5D_5N) δ (multiplicity, proton intergration, J in Hz)	^{13}C NMR DEPT (100 MHz, C_5D_5N) δ (multiplicity)	^{1}H–^{1}H COSY correlation (300 MHz, C_5D_5N)
1	13.06 (br s, 1H)	165.13 (s)	—
2	—	152.03 (s)	—
3	8.11 (s, 1H)	136.07 (d)	3-H, 10-H_3
4	—	110.61 (s)	—
5	6.97 (t, 1H, J = 6.8)	85.56 (d)[b]	5-H, 6-H_2
6	2.65 (m, 2H)	41.45 (t)	6-H_2, 7-H
7	5.02 (br s, 1H)	71.62 (d)[b]	7-H, 8-H
8	4.46 (br s, 1H)	88.94 (d)	8-H, 9-H_2
9	4.18 (dt, 2H, J = 8, 16)	62.49 (t)	9-H_2, 8-H
10	1.87 (s, 3H)	12.02 (q)	10-H_3, 3-H

[a]PMR spectral assignments made are based on ^{1}H–^{1}H COSY.
[b]Assignments may be interchangeable.

showed diagonal peaks corresponding to the signals 6-H_2 at δ 2.65 with those of 5-H and 7-H at δ 6.97 and 5.02, respectively, indicating that the nonoxygenated methylene protons are coupled with the two oxygenated methine protons of the pyran moiety. The COSY spectrum also showed the coupling between oxygenated methine proton (7-H) at δ 5.02 and oxygenated methine proton (8-H) at δ 4.46. The coupling interaction also observed between methylene protons at δ 4.18 (9-H_2) and methine proton at δ 4.46 (8-H). Thus all the coupling interactions of the protons on the substituted pyran were observed. The allylic coupling corresponding to the protons of the α,β-unsaturated acid side chain was noted between 10-H_3 at δ 1.87 and 3-H at δ 8.11.

The proposed structure was confirmed further by mass spectral fragmentation data. EIMS and CIMS of **6** produced a weak molecular ion at m/z 248 (M^+) along with ions at m/z 212 (M^+-2H_2O), 197 (M^+-2H_2O, CH_3) and 153 (M^+-2H_2O, CH_3, CO_2). The EIMS and CIMS showed the fragment ion at m/z 127 (M^+-α,β-unsaturated acid side chain, $2H_2O$) indicating that the tetrahydropyran moiety is substituted by the α,β-unsaturated acid side chain. The FABMS and EIMS of the acetyl derivative of the compound showed fragment ions without a molecular ion at m/z 329 (M^+-CO_2H), 327 (M^+-CO_2H, 2H) and the ion at m/z 201 ($M^+-3COCH_3$, CO_2). The fragment ions at m/z 81, 83 also indicated the presence of tetrahydropyran moiety. Based on the spectral data, **6** was characterised as 2-methyl-3-(4,5,7-trihydroxy-8-hydroxymethyltetrahydro-6H-4-pyranyl)-2-propenoic acid. The stereochemistry of the chiral centers could not be assigned based on the available data and the X-ray crystallographic studies would clarify the stereochemical aspects which is in progress.

Biology

In performing preliminary tests for pharmacological activity in mice, 5-octadecylpyrrole-2-carboxaldehyde (**1**) did not produce any significant changes in the autonomic, behavioural or neurological responses up to doses of 300 mg/kg body wt when administered orally within 4 h. The changes in the behavioural, autonomic and neurological responses observed within 3–5 h, only when the doses of 600 and 1000 mg/kg body wt were given orally may be due to some toxic symptoms (convulsions, tremors, respiratory distress and death by respiratory and circulatory failure). The LD_{50} (oral) for **1** was found to be approximately 300 mg/kg body wt. While screening for antimicrobiological activities, no significant antibacterial, antifungal or antiviral activity was found for **1**, **2**, **5** and **6**.

In studying the effect of **1** for hypoglycemic activity in normal rats, significant reduction in blood glucose levels observed at a dose of 30 mg/kg body wt/oral when compared to matching controls treated with the vehicle, i.e. gum acacia mucilage. The blood glucose reduction observed with the above dose was found to be equivalent to 30 μg/kg body wt dose of glibenclamide administered orally. Similar pattern was observed in rats made diabetic with alloxan treatment. In diabetic rats also, **1** produced hypoglycemic activity and quantitatively the effect produced by the dose of 30 mg/kg body wt/oral was equivalent to dose 30 μg/kg body wt of glibenclamide administered orally. The compound also produced significant hypoglycemic activity in alloxan induced diabetic rats even at lower dose level, i.e. 10 mg/kg body wt/oral when compared to matching control and glibenclamide (30 μg/kg body wt/oral) treated rats. The results of the antidiabetic activity are given in the Table 4 and Figure 1. Compound **1** produced hypoglycemic activity in normal as well as alloxan induced diabetic rats on oral administration, it appears to be absorbed orally and produce antidiabetic activity by pancreatic or extrapancreatic mechanisms. Since the LD_{50} of **1** is found to be approximately 300 mg/kg body wt/oral (in mice) and it produces considerable antidiabetic activity with 30 mg/kg body wt/oral, it is worth conducting both toxicity and antidiabetic studies in depth. Also, the presently available oral antidiabetic drugs are known to act by extrapancreatic mechanisms by way of increasing tissue utilisation of glucose and none of these drugs are capable of regenerating pancreas to synthesise insulin. It is also worth studying the long term effects of **1** on the pancreatic mechanisms to find out whether it is capable of regenerating pancreatic tissues to synthesise insulin.

Table 4. The effect of 5-octadecylpyrrole-2-carboxaldehyde on blood glucose levels in normal and diabetic rats (route: oral)

Group	Time interval															
	0 h		2 h			4 h			6 h			8 h				
	Average blood glucose (mg/dL)	Average % of blood glucose reduction ±SEM	Average blood glucose (mg/dL)	Average % of blood glucose reduction ±SEM	P^a	P^b	Average blood glucose (mg/dL)	Average % of blood glucose reduction ±SEM	P^a	P^b	Average blood glucose (mg/dL)	Average % of blood glucose reduction ±SEM	P^a	P^b	Average blood glucose (mg/dL)	Average % of blood glucose reduction ±SEM	P^a	P^b
Normal control	87.98	0	85.50	2.81 ± 0.61	—	—	85.27	3.01 ± 0.87	—	—	82.33	6.42 ± 1.52	—	—	74.41	15.40 ± 0.64	—	—
Normal ODPC[c] 30 mg/kg	83.80	0	48.90	44.27 ± 8.32	0.001	0.028	47.71	43.28 ± 5.69	0.0001	0.03	40.20	54.40 ± 7.45	0.0002	0.09	43.70	50.12 ± 7.55	0.001	0.2
Diabetic control	210.81	0	209.45	0.65 ± 0.20	—	—	205.16	2.68 ± 0.83	—	—	196.74	5.95 ± 1.30	—	—	189.32	10.75 ± 1.49	—	—
Diabetic ODPC 10 mg/kg	304.01	0	292.32	3.88 ± 0.72	0.003	0.007	212.78	29.99 ± 0.40	0	0.11	208.44	31.22 ± 0.83	0.596×10^{-7}	0.18	203.54	33.18 ± 0.64	0.59×10^{-7}	0.3
Diabetic ODPC 30 mg/kg	267.40	0	177.70	34.09 ± 2.76	0.17×10^{-5}	0.006	174.30	35.20 ± 2.35	0.77×10^{-6}	0.004	172.6	36.06 ± 2.08	0.101×10^{-5}	0.009	144.3	46.63 ± 3.18	0.53×10^{-5}	0.1
Normal glibenclamide 30 μg/kg	94.26	0	85.33	12.23 ± 1.81	0.9×10^{-4}	—	72.93	22.56 ± 1.06	0	—	65.22	30.80 ± 3.66	0.003	—	59.76	35.48 ± 3.45	0.0002	—
Diabetic glibenclamide 30 μg/kg	212.66	0	180.66	14.94 ± 4.31	0.003	—	166.66	21.24 ± 7.29	0.0142	—	149.55	29.69 ± 4.26	0.008	—	133.33	36.76 ± 5.23	0.0009	—

[a] p, Compared with vehicle treatment ($n = 6$).
[b] p, Compared with 30 μg/kg body wt./oral of glibenclamide ($n = 6$).
[c] ODPC = 5-octadecylpyrrole-2-carboxaldehyde.

Experimental

All the melting points were determined on Automelpo HMK dreader hot plate and are uncorrected. The ^1H and ^{13}C NMR spectra were recorded on 400 MHz (Bruker FT WM-400/Unity Varian Vista), 300 MHz (Bruker FT DRX-300), 200 MHz (Gemini) or 90 MHz (Jeol JNM FX-90Q) spectrometers with tetramethylsilane as internal standard. EIMS and FABMS were taken on Jeol JMS-D300 and Jeol JMS-SX102 spectrometers, respectively. Elemental analysis carried on Carlo Erba 1108 analyser. Optical rotations were measured on a Rudolf Autopol III polarimeter. The IR spectra were taken on Perkin–Elmer (FTIR) 1800.

Extraction and isolation

The organism was collected during September 1995 on the coasts of Vizhinjam near Trivandrum (8° 41′ N, 76° 51′ E) at a depth of 10–15 m. The specimen was identified as *M. mytilorum* by the courtesy of Dr. P. A. Thomas, Central Marine Fisheries Research Institute, Vizhinjam, Trivandrum, India. The organism was extracted with methanol at room temperature at an interval of every 48 h. The combined extracts were concentrated under reduced pressure. Thus the residue obtained was repeatedly digested with ethyl acetate and the ethyl acetate soluble fraction was concentrated under vacuum to yield crude dark brown coloured gummy residue (25 g). Weight of the air dried organism after extraction was 2.0 kg. The ethyl acetate soluble fraction of the methanolic extraction was chromatographed over silica gel column using eluants with increasing polarity starting from hexane through ethyl acetate to methanol. Fractions were collected and monitored through silica gel TLC and a total of eight fractions (I–VIII) were collected. Fraction I (*n*–hexane) did not show any distinct spots on silica gel TLC and no further investigation was carried. Fraction II (3–5% EtOAc–hexane) on rechromatography over silica gel column using hexane and hexane–ethyl acetate (1–2% EtOAc–hexane) as eluants yielded a mixture from the later. The mixture on further chromatography (3% EtOAc–hexane) over 2% silver nitrate impregnated silica gel column yielded pale yellow coloured flakes, **1** (1.7 g) and **2** (15 mg) from the initial and later fractions respectively. Fraction III (10–20% EtOAc–hexane) gave positive Liebermann-Burchard test for sterols and preliminary spectral data indicated that the fraction is a mixture of monohydroxy sterols and no further investigation was carried. Fraction IV (30–40% EtOAc–hexane) on crystallisation from 5% EtOAc in hexane followed by washing the solid on the filter paper with acetone yielded colourless flakes, **3** (50 mg). Fraction V (40–50% EtOAc–hexane) on crystallisation from EtOAc:hexane (80:20) yielded crystalline solid (30 mg) which, still contaminated with colouring matter, on rechromatography (50% EtOAc–hexane) over silica gel column yielded colourless crystalline solid, **4** (20 mg). Fraction VI (60% EtOAc–hexane) on crystallisation from EtOAc:hexane (1:1) yielded colourless amorphous solid, **5** (11 mg). Fraction VII (80% EtOAc–hexane) contained high percentage of intractable gum and no

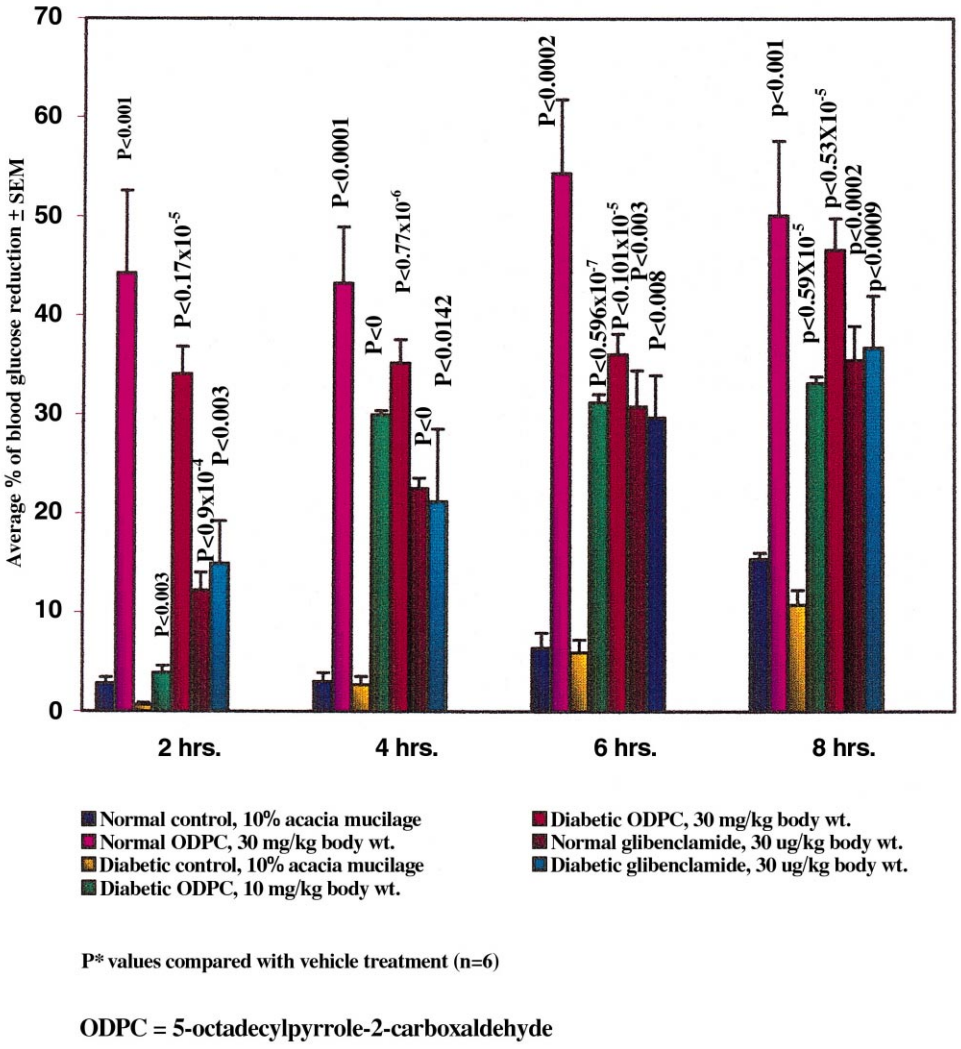


Figure 1. Hypoglycemic and antidiabetic activity of 5-octadecylpyrrole-2-carboxaldehyde in rats (route: oral).

further investigation was undertaken on this. Fraction VIII (EtOAc) on crystallisation from EtOAc:MeOH (95:5) gave crystalline needles, **6** (60 mg).

5-Octadecylpyrrole-2-carboxaldehyde (1). Pale yellow flakes, mp 56–58 °C, UV λ_{max}^{EtOH} nm (ϵ): 302 (16,000); IR ν_{max}^{KBr}: 3290, 3040, 2910, 2820, 1640, 1610, 1490, 1460, 1395, 1340, 1190, 1040, 780, 715 cm^{-1}; EIMS *m/z* (% rel. int.) 347 (M^+, 3), 332 (7.1), 318 (18), 304 (86.7), 290 (16.2), 276 (10.2), 263 (12), 262 (8), 249 (11.7), 235 (6.9), 221 (8.3), 207 (6), 193 (7), 178 (10.9), 164 (11.7), 150 (35.8), 136 (7.3), 122 (66), 108 (100), 96 (25), 94 (15), 80 (78), 55 (15), 42 (18). HREIMS *m/z*: found 347.2256 (M^+), calcd for $C_{23}H_{41}NO$, 347.2243. Anal. found C 79.58, H 11.80, N 4.00; calcd for $C_{23}H_{41}NO$, C 79.54, H 11.82, N 4.03. ^1H NMR, ^{13}C NMR DEPT and ^1H–^1H COSY spectral data, see Table 1.

(6′*Z*)-5-(6′-Heneicosenyl) pyrrole-2-carboxaldehyde (2). Pale yellow flakes, mp 40–43 °C, UV λ_{max}^{EtOH} nm (ϵ): 302 (16000); IR ν_{max}^{KBr}: 3300, 3040, 2910, 2840, 1642, 1610, 1470, 1390, 1350, 1260, 1190, 1040, 780, 720 cm^{-1}; EIMS *m/z* (% rel. int.) 387 (M^+, 2), 358 (5), 360 (5), 334 (6), 332 (5), 320 (10.5), 307 (8.5), 306 (42), 305 (5), 292 (5.7), 291 (5), 277 (9), 263 (7), 249 (5), 235 (5), 222 (5), 221 (4), 207 (3), 193 (1), 192 (1), 178 (8), 164 (11.6), 150 (25.3), 136 (30), 122 (84), 108 (88), 80 (100), 72 (15), 57 (28). HREIMS *m/z*: found 387.3599 (M^+), calcd for $C_{26}H_{45}NO$, 387.3612. Anal. found C 80.68, H 11.60, N 3.58; calcd for $C_{26}H_{45}NO$, C 80.62, H 11.63, N 3.62. ^1H NMR (90 MHz, $CDCl_3$) δ: 10.28 (1H, br s, NH), 9.29 (1H, s, CHO), 6.89 (1H, t, *J* = 3.1, 3-H), 6.07 (1H, t, *J* = 3.1, 4-H), 5.34 (2H, t, *J* = 5.0, 6′, 7′-H), 2.65 (2H, t, *J* = 7.1, 1′-H_2), 2.22 (2H, m, 5′-H_2), 2.01 (2H, m, 8′-H_2), 1.62 (4H, m, 2′, 9′-H_2), 1.25 (26H, br s), 0.89 (3H, t, *J* = 6.8, 21′-H_3). ^{13}C NMR DEPT (75 MHz, $CDCl_3$) δ: 178.1 (d, CHO), 143.5 (s, C-5), 131.8 (s, C-2), 129.7 (d, C-6′), 128.3 (d, C-7′), 122.8 (d, C-3), 109.4 (d, C-4), 31.9 (t, C-1′), 31.8 (t, C-5′, 8′), 29.9-27.2 (t, totally 14C), 22.7 (t, C-20′), 14.1 (q, C-21′).

Batylalcohol (3). Colourless flakes, mp 69–71 °C, $[\alpha]_D^{28}$ +2.58° (*c* = 0.1, $CHCl_3$). Its identification was confirmed by the comparison of the spectral data with those reported in the literature.[24]

***p*-Hydroxyphenylacetic acid (4).** Colourless crystals, mp 148–152 °C. Its identity was confirmed by the physical and spectral analysis.[25] UV λ_{max}^{EtOH} nm (ϵ): 278 (4500), 266 (3700), 232 (10500); IR ν_{max}^{KBr} 3400–3000, 2920, 2850, 1700, 1605, 1510, 1440, 1400, 1220, 1180, 895, 820, 785, 645 cm^{-1}; EIMS *m/z* (% rel. int.) 152 (M^+, 22), 107 (100), 77 (33), 51 (18); CIMS *m/z* (% rel. int.) 152 (M^+, 100), 107 (80). Anal. found C 63.14, H 5.26; calcd for $C_8H_8O_3$, C 63.16, H 5.26. ^1H NMR (C_5D_5N, 400 MHz) δ: 10.35 (2H, br s), 7.48 (2H, d, $J=7.7$ Hz), 7.19 (2H, d, $J=7.7$ Hz), 3.83 (2H, s).^{13}C NMR (C_5D_5N, 100 MHz) δ: 174.80, 158.00, 131.30 (2C), 126.71, 116.44 (2C), 41.62.

(2*S*,3*R*,4*E*)-1,3-Dihydroxy-2-[(heneicosanoyl) amino]-4-heneicosene (5). Colourless amorphous solid, mp 75–77 °C, $[\alpha]_D^{28}$ –11.0° ($c=0.1$, $CHCl_3$); IR ν_{max}^{KBr} 3500–3100, 2910–2840, 1640, 1610, 1545, 1470, 1045, 975 cm^{-1}; FABMS *m/z* (% rel. int.) 649 (M^+, 10), 647 (13), 633 (16), 631 (18), 619 (18), 605 (18), 591 (6), 368 (12.5), 340 (12.5), 309 (3), 310 (3), 296 (20), 278 (62), 264 (70), 252 (14), 239 (1), 225 (2), 211 (2.5), 197 (2.5), 183 (3), 169 (3.5), 155 (4), 141 (4), 127 (5), 113 (5), 99 (12), 97 (20), 95 (40), 85 (16), 81 (52), 71 (31), 69 (62), 57 (66), 55 (100), 43 (87), 29 (33); EIMS *m/z* (% rel. int.) 368 (13.6), 366 (15.5), 340 (13.7), 309 (3.5), 296 (3), 278 (4.6), 264 (5), 252 (5), 238 (1), 239 (2), 225 (3.5), 211 (3.5), 197 (3.5), 183 (3.7), 169 (3.9), 155 (4.7), 141 (6.7), 127 (10.2), 113 (8.9), 99 (13), 97 (35), 85 (43), 83 (53), 71 (65), 57 (100), 43 (79). Anal. found C 77.75, H 12.69, N 2.14; calcd for $C_{42}H_{83}NO_3$, C 77.66, H 12.79, N 2.16. ^1H NMR and ^{13}C NMR spectral data, see Table 2.

Acetylation of 5. To a solution of **5** (3 mg) in pyridine (1 mL), Ac_2O (0.5 mL) was added and the mixture was kept overnight at room temperature. The excess reagents were removed in vacuo and the residue was partitioned between water and ether. On evaporation of the solvent, it gave a residue which on chromatography over a small column of silica gel yielded diacetyl derivative (**5a**, 3 mg). **5a**: a white amorphous powder, mp 53–58 °C, $[\alpha]_D^{28}$ −13.5° ($c=0.1$, $CHCl_3$); IR ν_{max}^{KBr} 3340, 2910, 2855, 1740, 1642, 1610, 1545, 1475, 1040, 970 cm^{-1}; Anal. found C 75.25, H 11.91, N 1.90; calcd for $C_{46}H_{87}NO_5$, C 75.31, H 11.87, N 1.91. ^1H NMR (200 MHz, $CDCl_3$) δ: 5.8 (1H, dt, $J=14.1$, 6.4 Hz), 5.65 (1H, d, $J=9$ Hz), 5.58 (1H, dd, $J=14.1$, 6.4 Hz), 5.30 (1H, dd, $J=14.1$, 6.4 Hz), 4.62 (1H, m), 4.40 (1H, dd, $J=6$, 11.6 Hz), 4.10 (1H, dd, $J=3.9$,11.6 Hz), 2.25 (2H, t, $J=7.5$ Hz), 2.05 (6H, s), 1.98 (2H, m), 1.60 (2H, m), 1.25 (62H, br s), 0.88 (6H, t, $J=6.8$ Hz).

2-Methyl-3-(4,5,7-trihydroxy-8-hydroxymethyltetrahydro-6H-4-pyranyl)-2-propenoic acid (6). Colourless crystalline needles, mp 191–193 °C, $[\alpha]_D^{28}$ +26.33 ($c=0.3$, MeOH), UV λ_{max}^{EtOH} nm (ϵ): 219 (9500); IR ν_{max}^{KBr} 3600–3300, 3000, 1690, 1650, 1430, 1320, 1280, 1220, 1195, 1175, 1100, 1060, 1000, 950, 865, 848, 750, 728 cm^{-1}; EIMS *m/z* (% rel. int.) 248 (M^+, 1), 212 (3), 197 (4), 153 (7.2), 150 (4.2), 127 (43), 117 (100), 110 (66), 99 (43), 98 (12), 83 (10), 81 (7), 73 (66), 71 (24), 69 (18), 57 (6), 55 (20), 45 (54), 43 (31); CIMS *m/z* (% rel. int.) 248 (M^+, 2), 225 (8.6), 212 (3), 197 (3), 167 (5.8), 153 (20.2), 127 (45.7), 117 (17.2), 99 (15.6), 81 (100). Anal. found C 48.30, H 6.49; calcd for $C_{10}H_{16}O_7$, C 48.39, H 6.45. ^1H NMR, ^{13}C NMR DEPT and ^1H–^1H COSY spectral data, see Table 3.

Acetylation of 6. To a solutioon of **6** (5 mg) in pyridine (1 mL), Ac_2O (0.7 mL) was added and the mixture was kept overnight at room temperature. The excess reagents were removed in vacuo and the residue was partitioned between water and $CHCl_3$. On evaporation of the solvent, it gave a residue which on chromatography over a small column of silica gel yielded a triacetyl derivative (**6a**, 6 mg). **6a**: colourless crystalline solid, mp 86–90 °C; IR ν_{max}^{KBr} 3500–3350, 3150–2980, 2920, 2850, 1740–1695, 1645, 1430, 1320, 1275, 1195, 1175, 1060, 950, 865, 750 cm^{-1}; FABMS *m/z* (% rel. int.) 329 (M^+−COOH, 18), 327 (85), 201 (40), 153 (6), 127 (79), 107 (14), 89 (14), 83 (14), 81 (100), 69 (8), 65 (9), 63 (8), 55 (6.5), 51 (10), 43 (62), 31 (4.5); EIMS *m/z* (% rel. int.): 329 (3), 327 (5), 201 (7), 153 (5), 127 (11), 81 (100), 55 (7), 43 (77). Anal. found C 51.24, H 5.84; calcd for $C_{16}H_{22}O_{10}$, C 51.34, H 5.88. ^1H NMR (200 MHz, $CDCl_3$) δ: 9.93 (1H, s), 7.21 (1H, s), 6.21 (1H, t, $J=6.7$ Hz), 5.14 (1H, br d, $J=6.5$ Hz), 4.25 (2H, m), 4.15 (1H, br s), 2.45 (1H, dd, $J=6.5$, 14.0 Hz), 2.2 (1H, dd, $J=6.5$, 14.0 Hz), 2.02 (9H, s), 1.86 (3H, s).

Acute toxicity studies and preliminary tests of 1 for pharmacological activity (blind screening studies)[26]

Swiss albino mice of male sex, weighing 20–22 g were obtained from the animal house, Ghosh Enterprise, Calcutta, India. They were housed in standard conditions and received standard diet and water ad libitum. The animals were divided into various groups (each of six animals). The test compound was suspended in 10% gum acacia and the suspension was administered orally to each group of animals in doses 100, 300, 600 and 1000 mg/kg body weight and the control group received only equal volume of 10% gum acacia mucilage. The animals were observed for 4 h after administration of the test compound for any behavioural, autonomic and neurological responses following Irwin's method.[27] Animals were also observed for any toxic symptoms and mortality during the first 24 h of the post treatment of the test compound.

Antibacterial and antifungal activity

Compounds **1, 2, 5** and **6** were studied for the antibacterial (against 3 Gram-positive bacteria, viz. *Bacillus pumilis*, *Bacillus subtilis* and *Staphylococcus aureus* and 2 Gram-negative bacteria, viz. *Escherichia coli* and *Pseudomonas aeruginosa)* and antifungal (against *Candida albicans*, *Aspergillus niger* and *Rhizopus nigricans)* activities. They were tested for the determination of inhibition zone by the cup-diffusion method.[28,29]

Antiviral studies[30]

Antihepatitis-B virus properties by HBsAg binding studies. Hepatitis-B surface antigen binding studies were carried out as the first part of the antiviral studies for

the compounds **1**, **2**, **5** and **6**. Equal volume of HBsAg positive plasma and the test compound (2 mg/mL) was added and incubated at 37 °C for a period of 5 days. The binding effect of these compounds which equates with *in vitro* inactivation of HBsAg was analysed by ELISA procedure conducted every day.

Anti Herpes simplex virus (HSV) properties using Vero cell line[30]

Preparation of the compounds for anti HSV studies. The anti HSV studies were carried out for the compounds **1**, **2**, **5** and **6**. The test compound was dissolved in DMSO (dimethylsulphoxide) and centrifuged at 2000 rpm for 10 min. The supernatant was removed and filtered in a membrane filter with porosity of 0.2 micron. The filtrate was used for the antiviral studies against HSV-1.

Study protocol. Vero cell monolayers were used for the anti HSV-1 evaluatory studies. Four sets of six Vero cell culture tubes were used. Group A which receives 0.1 mL each of sterile DMSO acted as DMSO control. Group B tubes received 0.1 mL each of the test compound, 2 mg/mL, acted as compound control. Group C tubes were inoculated with 0.1 mL of AC strain of HSV-1 at a concentration of 10 pfu/mL (positive virus control) and Group D also received the same as Group C. All the tubes were incubated at room temperature for 90 min. After incubation, Group D tubes alone were inoculated with 0.1 mL of test compound at a concentration 2 mg/mL (test group). Two mL Eugles maintenance MEM was added to all the tubes and incubated at 37°C for 2 weeks. They were observed every day for any evidence of cytopathic effect by HSV-1 and cytotoxicity by the compound itself.

Hypoglycemic and antidiabetic activity of 1

Animals. The Wistar albino rats of male sex weighing 100–140 g supplied by B.N. Ghosh Enterprise, Calcutta, India were used for the study. They were housed in standard conditions and received a standard diet and water ad libitum. The animals were divided into normal test, normal control, diabetic test, diabetic control, and normal and diabetic standard groups of six rats each.

Induction of diabetes and biochemical analysis of blood glucose in normal and diabetic rats. Single dose of alloxan monohydrate (150 mg/kg body wt) as 10% saline solution was administered subcutaneously to rats under the experiment. Diabetes was confirmed by the rise in blood glucose and the glucose levels were estimated using Nelson Somogyi's method.[31,32] The diabetic rats with stable blood glucose levels (above 200 mg/dL) were only included in the experiment.

In the hypoglycemic study the test compound suspension at the dose of 30 mg/kg body wt in 10% gum acacia was given orally to the group of normal rats under test. Simultaneously the normal control group rats received only 10% gum acacia mucilage. In the antidiabetic study, the test compound suspension at the doses 10 and 30 mg/kg body wt in 10% gum acacia was administered orally to the groups of diabetic rats under test. Control diabetic rats received only 10% gum acacia mucilage. The rats under the groups, normal standard and diabetic standard received glibenclamide suspension in 10% acacia at a dose level of 30 μg/kg body wt orally. Blood samples were collected at 0, 2, 6 and 8 h intervals and subjected to the assay of fasting glucose.[31,32] Analysis of the experimental data was carried out by employing student *t*-test.[33,34]

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