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# THREE NOVEL CYCLIC AMIDES FROM *CLAUSENA LANSIUM*

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**Key Word Index**—*Clausena lansium*; Rutaceae; amides; clausenamide; neoclausenamide; cycloclausenamide.

**Abstract**—Three novel cyclic amides, clausenamide, neoclausenamide and cycloclausenamide, that lower elevated blood SGPT levels have been isolated from the leaves of *Clausena lansium*. By means of spectrometric data combined with chemical reactions, their structures have been elucidated. The proposed structures of clausenamide and cycloclausenamide were further confirmed by X-ray crystallographic analysis.

## INTRODUCTION

*Clausena lansium* (Lour.) Skeels, a fruit tree, is widely distributed in the southern part of China [1]. In folk medicine, the boiled water extract of its leaves is used for the treatment of certain dermatological diseases and of acute and chronic viral hepatitis. Heptaphylline and dehydroindicolatone [2] and lansamide-I [3] have been isolated from the roots and leaves of the plant. We have now investigated the leaf extract because work in our Institute had shown that this extract lowers elevated blood SGPT levels [4].

## RESULTS AND DISCUSSION

From the boiling-water extract of the dried leaves of *C. lansium* three novel crystalline cyclic amides clausenamide (**1**), Neoclausenamide (**2**) and cycloclausenamide (**3**) were isolated.

Clausenamide (**1**) had a molecular formula $C_{18}H_{19}NO_3$ from high resolution mass spectrometry 297.1364 $[M]^+$ and elemental analysis. The IR spectrum suggested the presence of hydroxy groups (3400, 3310 $cm^{-1}$), an amide carbonyl group (1680 $cm^{-1}$) and monosubstituted benzene rings (1600, 1580, 1490, 1450, 740, 690 $cm^{-1}$). The benzenoid nature was supported by the UV spectrum $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 257 (2.70). The compound was negative to ferric chloride, 2,4-dinitrophenylhydrazine and bromine water indicating the absence of phenolic or enolic hydroxy groups, ketones or aldehydes and double bonds. However, after reaction with periodic acid, a positive reaction with 2,4-dinitrophenylhydrazine was given.

Clausenamide (**1**) gave a diacetate (**1a**) with acetic anhydride–pyridine and reduction with lithium aluminium hydride gave an amine, deoxyclausenamide (**1b**), with a $[M]^+$ 14 mass units less than that of (**1**), corresponding to the reduction of a lactam carbonyl to a methylene group. The amine (**1b**) was further characterized as the acetyl deoxyclausenamide (**1c**).

On the basis of these observations and from the $^1H$ NMR (Table 1), $^{13}C$ NMR (Table 2) and EI mass spectrum of (**1**) the structure 3-hydroxy-4-phenyl-5 (1-hydroxy-1-phenyl methyl)-1-methyl-2-pyrolidinone was proposed. Further support for this structure was given by hydrogenolysis of the α-benzyl acetate group in acetyl clausenamide (**1a**) to the desoxy compound acetyl dehydroxyclausenamide (**1e**). Chromic acid treatment [5] of (**1**) gave the expected oxidation of a benzyl alcohol to a phenyl ketone (IR 1690 $cm^{-1}$) and the product, clausenamidone (**1d**), also had an IR band at 1670 $cm^{-1}$ (amide).

Acid hydrolysis of clausenamide (**1**) failed to yield the expected δ-lactone. With 12 N HCl–EtOH (1:1) (**1**) yielded anhydroclausenamide (**1g**) presumably via either a 1,4-hydride shift or via consecutive 1,2-hydride shifts. With 12 N HCl–water (1:1) (**1**) yielded anhydroclausenamide (**1g**) and anhydrocycloclausenamide (**1i**) presumably from an intermediate carbonium ion. Anhydrocycloclausenamide (**1i**) can only be formed if the H-4 and H-5 in (**1**) are *cis*-orientated.

Clausenamide (**1**) was expected to be a racemate since neither it nor the derivatives (**1f**) and (**1d**) exhibited optical activity. An X-ray crystal analysis [6] confirmed the proposed structure and showed that clausenamide (**1**) was a racemate with configurations 3*R*, 4*S*, 5*S*, 7*R* and 3*S*, 4*R*, 5*R*, 7*S* (Fig. 1).

Neoclausenamide (**2**) had the same molecular formula and UV spectrum as (**1**) and also gave a very similar EI mass spectrum. However, it was separated from (**1**) by HPLC and TLC and had different IR and NMR spectra (Tables 1 and 2). Neoclausenamide (**2**) gave a diacetyl derivative (**2a**) with the same molecular formula and a very similar EI mass spectrum as acetyl clausenamide (**1a**). Comparison of the $^1H$ NMR spectrum (Table 1) of (**2**) with that of (**1**) indicated that the chemical shifts of H-3 in these two compounds were very similar, but H-4 and H-5 in (**2**) (δ 3.07, 1H, *t*, $J$ = 7 Hz and δ 3.89, 2H, *m*) were now in the *trans*-configuration as compared with the *cis*-configuration for (**1**) H-4 and H-5 (3.50, 1H, *dd*, $J$ = 10, 8 Hz and δ 4.30, 1H, *dd*, $J$ = 8, 2 Hz). Treatment of (**2**) with 12 N HCl–water (1:1) gave only anhydroclausenamide (**1g**) and not anhydrocycloclausenamide (**1i**) which was in agreement with the proposal that H-4 and H-5 are required for the formation of (**1i**). Thus, structure (**2**) was proposed for neoclausenamide.

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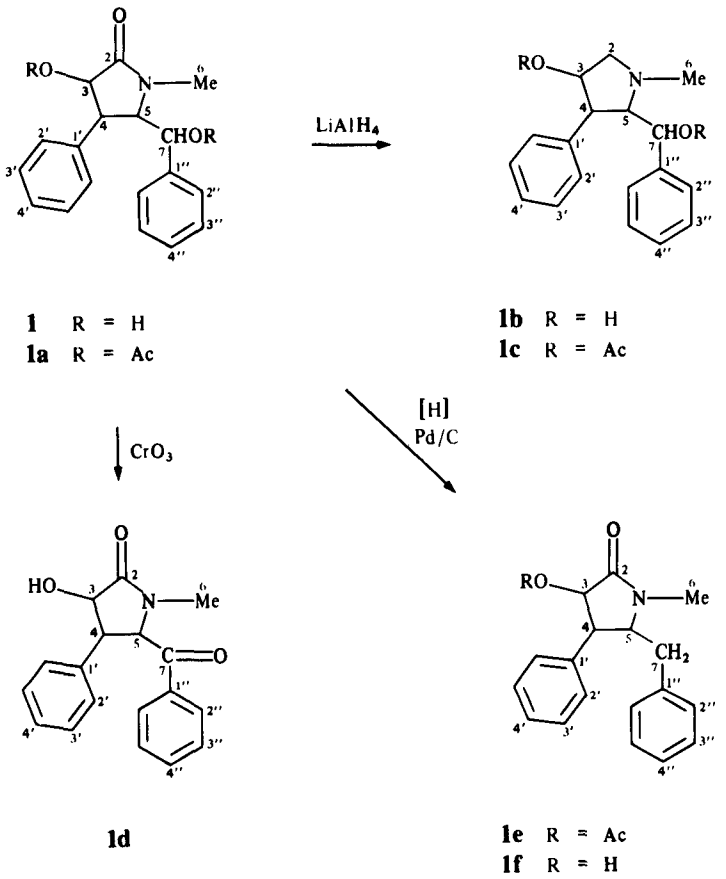
Table 1. $^1$H NMR spectral data of **1–3** (TMS as internal standard; 90 MHz, $CDCl_3$ if no exception is marked)

| | **1*** | **1a†** | **1c†** | **1d** | **1g** | **1h** | **1i** | **1j** | **2‡** | **2a** | **2b** | **3†** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| H-2α | | | 2.44 *dd* | | | | | | | | | |
| H-2β | | | 3.80 *dd* | | | | | | | | | |
| H-3 | 3.90 *d* | 5.60 *d* | 5.09 *m* | 5.40 *d* | | | | 5.30 *d* | 3.89 *m* | 5.25 *d* | 4.50 *d* | 4.81 *s* |
| H-4 | 3.50 *dd* | 4.07 *dd* | 3.53 *dd* | 3.86 *t* | | | | 4.34 *dd* | 3.07 *t* | 3.29 *t* | 3.30 *t* | 3.60 *s* |
| H-5 | 4.30 *dd* | 4.30 *dd* | 3.36 *t* | 4.95 *d* | 4.62 *t* | 4.60 *t* | | 4.00 *dd* | 3.89 *m* | 3.92 *t* | 5.60 *d* | 4.09 *s* |
| H-6 | 3.05 *s* | 2.86 *s* | 2.06 *s* | 2.92 *s* | 3.06 *s* | 3.02 *s* | 2.96 *s* | 3.09 *s* | 2.90 *s* | 2.95 *s* | 2.98 *s* | 2.95 *s* |
| H-7 | 4.65 *d* | 5.80 *d* | 5.36 *d* | | 7α 2.95 *dd* | 7α 2.85 *dd* | | 4.51 *d* | 5.00 *dd* | 6.23 *d* | | 5.00 *s* |
| | | | | | 7β 3.26 *dd* | 7β 3.20 *dd* | | | | | | |
| OH | 4.70–5.40 *m* | | | 2.01 *br* | 6.40 *br* | | 2.57 *s* | | 3-OH 5.53 *d* | | 2.41 *br* | |
| | (2H) | | | (1H) | (1H) | | (1H) | | 7-OH 5.73 *d* | | (2H) | |
| aromatic H | 6.50–6.70 *m* | 6.78–6.90 *m* | 7.20–7.44 *m* | 6.95–7.70 *m* | 6.70–7.70 *m* | 6.70–7.60 *m* | 6.90–7.55 *m* | 7.00–7.80 *m* | 6.75–6.93 *m* | 6.80–6.90 *m* | 7.20–7.80, | 7.10–7.50 *m* |
| | (2H) | (2H) | (10H) | (10H) | (10H) | (10H) | (9H) | (9H) | (2H) | (12H) | (10H) | (10H) |
| | 6.90–7.30 *m* | 7.10–7.40 *m* | | | | | | | 6.95–7.33 *m* | 7.10–7.35 *m* | | |
| | (8H) | (8H) | | | | | | | (8H) | (8H) | | |
| $CH_3COO$ | | 1.88 *s* | 1.71 *s* | | | | | 2.22 *s* | | 2.15 *s* | | |
| | | 2.04 *s* | 1.86 *s* | | | | | | | 2.20 *s* | | |
| $CH_3O$ | | | | | | 3.88 *s* | | | | | | |
| | | | | | | | 3.99 *m* (1H) | | | | | |
| | | | | | | | 4.36 *m* (3H) | | | | | |

*90 MHz, DMSO-$d_6$.
†200 MHz, $CDCl_3$.
‡200 MHz, DMSO-$d_6$.
*J* (Hz): Compound **1**: 3,4 = 10; 4,5 = 8; 5,7 = 2; **1a**: 3,4 = 11; 4,5 = 8; 5,7 = 4; **1c**: 2α,2β = 10; 3,4 = 4; 4,5 = 8; 5,7 = 8; **1d**: 3,4 = 8; 4,5 = 8; **1g**: 5,7 = 4; 7α,7β = 12; **1h**: 5,7 = 4; 7α,7β = 12; **ij**: 3,4 = 3; 4,5 = 7; 5,7 = 2; **2**: 3,4 = 7; 4,5 = 7; 5,7 = 3; 7-H, 7-OH = 5; 3-H, 3-OH = 7; **2a**: 3,4 = 4; 4,5 = 4; 5,7 = 4; **2b**: 3,4 = 5; 4,5 = 5

Table 2. $^{13}C$ NMR spectral data of **1**–**3** (90 MHz, TMS as internal standard)

| C | **1** (DMSO-$d_6$) | **1g** ($CDCl_3$) | **1i** ($CDCl_3$) | **2** (DMSO-$d_6$) | **3** ($CDCl_3$) |
|---|---|---|---|---|---|
| 2 | 173.6 *s* | 167.4 *s* | 174.6 *s* | 172.7 *s* | 172.2 *s* |
| 3 | 68.9 *d* | 142.8 *s* | 75.6 *d* | 69.3 *d* | 80.3 *d* |
| 4 | 49.9 *d* | 120.9 *s* | 55.9 *d* | 46.7 *d* | 50.7 *d* |
| 5 | 65.3 *d* | 60.3 *d* | 72.2 *d* | 68.4 *d* | 70.1 *d* |
| 6 | 30.4 *q* | 28.8 *q* | 29.0 *q* | 28.2 *q* | 27.3 *q* |
| 7 | 71.9 *d* | 36.8 *t* | 51.5 *d* | 77.3 *d* | 82.5 *d* |
| 1′ | 136.0 *s* | 131.8 *s* | 134.9 *s* | 140.5 *s* | 133.3 *s* |
| 2′ | | | 143.4 *s* | | |
| 1″ | 140.5 *s* | 135.1 *s* | 141.6 *s* | 141.8 *s* | 139.1 *s* |
| aromatic | 126.3–128.9 *m* | 126.8–129.1 *m* | 124.9–128.9 *m* | 125.9–128.5 *m* | 125.4–128.5 *m* |




**1** R = H
**1a** R = Ac

**1b** R = H
**1c** R = Ac

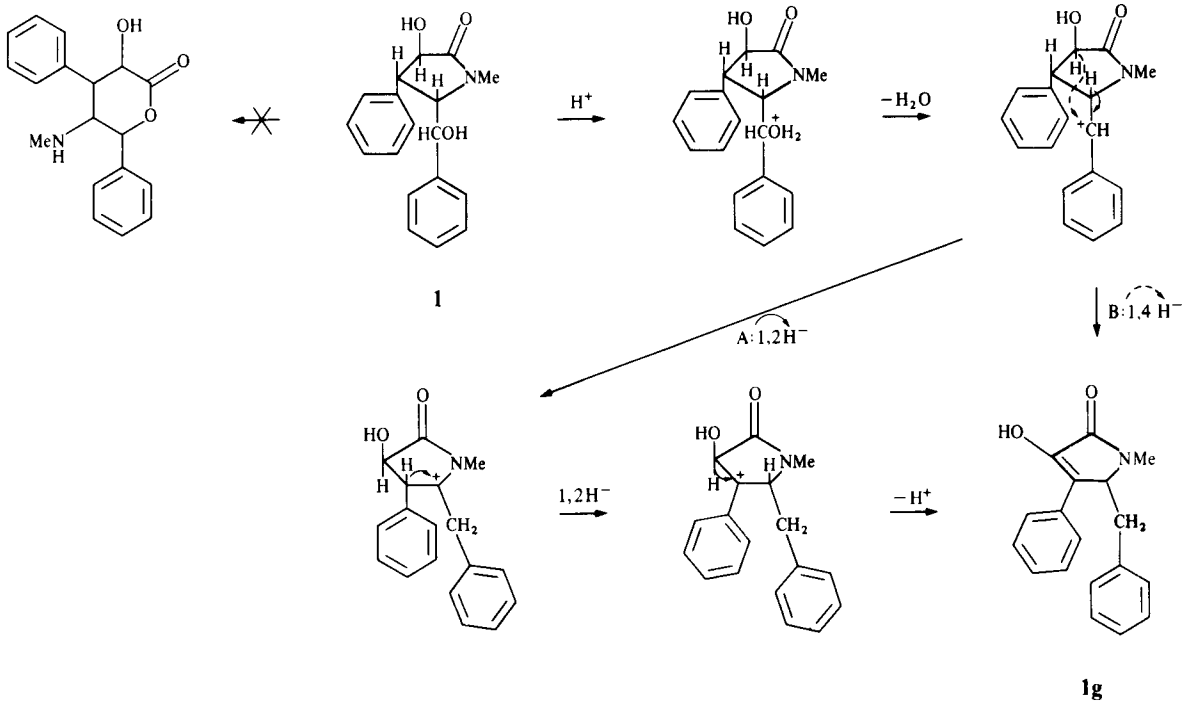
**1d**

**1e** R = Ac
**1f** R = H

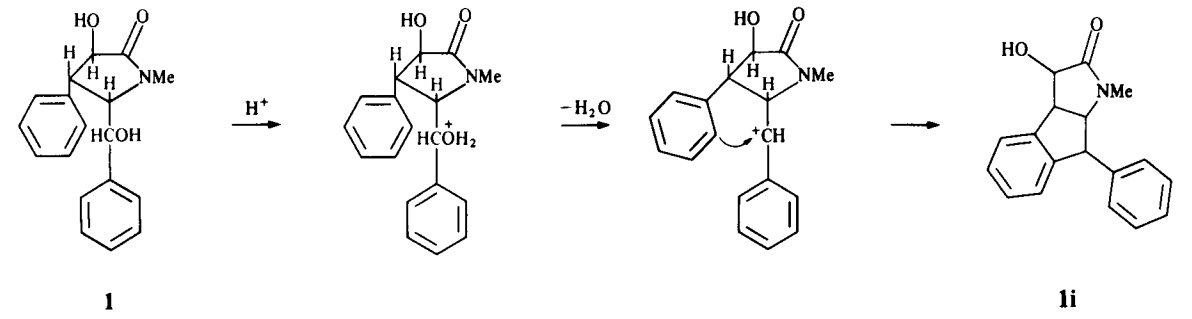
Oxidation of (**2**) with chromium trioxide gave neoclausenamidone (**2b**) which could be distinguished from clausenamide (**1d**) by $^1H$ NMR (Table 1) and IR spectroscopy. When either (**1d**) or (**2b**) was refluxed in 5% NaOH–MeOH it gave mixture of (**1d**) and (**2b**) in the proportion 1:4 regardless of which starting material was used. This is consistent with the proposal that neoclausenamide (**2**) possessed the more stable *trans* H-4 and H-5. Neoclausenamide (**2**) was expected to be a racemate. The absolute configuration of C-7 is still under investigation.

Cycloclausenamide (**3**) had a molecular formula $C_{18}H_{17}NO_2$ from HR mass spectroscopy 280.1371 $[M+1]^+$ and elemental analysis and it was optically active, $[\alpha]_D^{24.5} -40°$ (MeOH, *c* 0.225). The IR spectrum indicated the absence of a hydroxy group and the presence of an amide carbonyl group (1690 $cm^{-1}$) and benzene rings (3080, 3060, 3010, 1600, 1500, 750, 730, 700 $cm^{-1}$). The UV spectrum $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 257 (2.60) also supported a benzenoid assignment. The $^1H$ NMR spectrum (Table 1) had signals at $\delta$ 7.1–7.5 for 10 aromatic protons, a three protons singlet at $\delta$ 2.95 (N-Me) and four one-proton singlets at $\delta$ 3.6, 4.09, 4.80 and 5.0. The $^{13}C$ NMR spectrum had signals for 12 aromatic carbons, one N-Me, one carbonyl group and four tertiary carbon doublets.

It was calculated from the molecular formula of **3** that



**1**



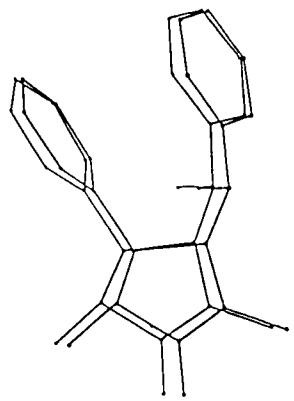
**1g**



**1**

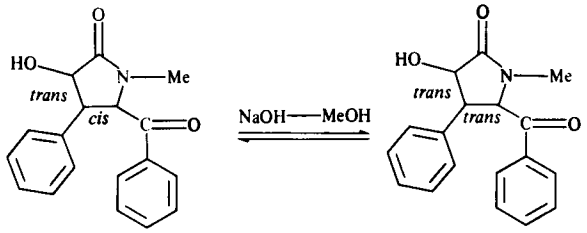


**1i**



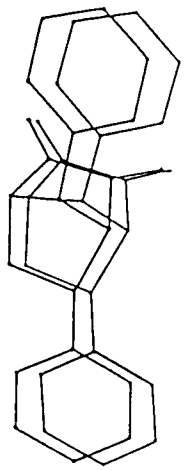
X - ray crystal structure of clausenamide **1**

Fig. 1.



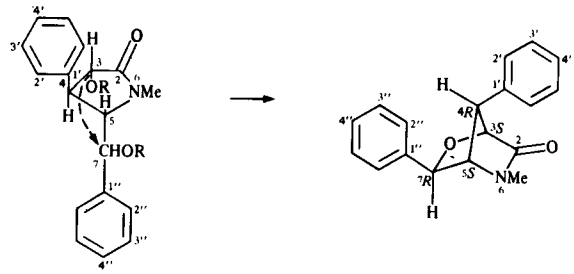
**1d** **2b**

if, as was indicated from spectroscopy of the material, two benzene rings and one amide carbonyl group were present, then two more rings must be present. An ether linkage seemed most likely for the unassigned oxygen atom. Structures which satisfied the criteria recorded could be formally derived by elimination of water between the diols at C-3 and C-7 of either clausenamide (**1**) or neoclausenamide (**2**) to form the bicyclic ethers. All of the data obtained for cycloclausenamide were in accordance with the structure (**3**) derived from neoclausenamide (**2**). The absence of coupling between protons on C-3, C-4, C-5 and C-7 observed for cycloclausenamide was presumably due to the angles between these adjacent protons being close to 90° [7]. The configuration at C-4 could not be determined from the physical data. However, an X-ray crystallographic analysis not only confirmed the proposed structure, but showed that cycloclausenamide had the 4R configuration [6] (Fig. 2).



X - ray crystal structure of cycloclausenamide **3**

Fig. 2.



**2** R = H
**2a** R = Ac

**3**

Since clausenamide and neoclausenamide were racemates, it seems probable that they are artefacts, but further work would be necessary to investigate their precursors.

## EXPERIMENTAL

Mps are uncorr. NMR spectra were recorded at either 90 or 200 MHz, with TMS as int. ref. HPLC was carried out on Partisil-10 ODS (250 × 4.6 mm) columns with UV detection at 254 nm. CC was carried out on silica gel (100–200 mesh, Qingdao), TLC on silica gel GF 254; detection was with modified Dragendorff's and Wagner reagents. A polyvinyl sulphonic acid ion exchange resin (H-form, cross linking 1 × 1, Nankai University) was used.

*Plant material* was collected from Yishan county, Guanxi province, China and was taxonomically examined by Assoc. Prof. Zhu Zhao-yi of this Institute.

*Extraction and isolation.* Dried leaves were boiled with $H_2O$ and the aq. extract concd to give a crude syrup. A portion of this syrup was mixed with silica gel and extracted with $CHCl_3$. The $CHCl_3$ extract was evapd and the residue washed with MeOH to give a yellow powder which was recrystallized × 3 from MeOH to give clausenamide (**1**) (yield 0.38% of dry wt). A second portion of the crude syrup was dissolved in 0.06 N HCl and the supernatant passed through a column of wet H-form cation ion exchange resin. The resin was then washed with deionized $H_2O$, air-dried, mixed with 2% $NH_4OH$ and finally extracted with $Et_2O$. The conc $Et_2O$ extract was chromatographed on a silica gel column in $CHCl_3$ and the sepd fractions recrystallized from MeOH to give neoclausenamide (**2**) and cycloclausenamide (**3**) (yield 0.041% and 0.022% dry wt, respectively).

*Clausenamide* (**1**). White needles, mp 239–240°; $[\alpha]_D^{21°}$ 0.00 (MeOH, *c* 0.53); Found: C 72.55, H 6.43, N 4.37%, calc. for $C_{18}H_{19}NO_3$: C 72.76, H 6.45, N 4.72%. EIMS *m/z* (rel. int.): 298 $[M+1]^+$ (0.18), 191 (65), 190 (100), 174 (18), 162 (38), 144 (7), 134 (60), 133 (38), 119 (19), 107 (7), 105 (15), 91 (24), 77 (29).

*Acetyl clausenamide* (**1a**). White cubes, mp 165–167°; Found: C 69.12, H 6.04, N 3.68%, calc. for $C_{22}H_{23}NO_5$: C 69.29, H 6.04, N 3.67%; IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1735, 1715 (shoulder), 1700, 1215; EIMS *m/z* (rel. int.): 382 $[M+1]^+$ (0.5), 261 (0.3), 232 (17), 172 (100), 144 (9), 91 (8), 43 (40).

*Deoxyclausenamide* (**1b**). White needles, mp 180–183°; IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3440, 3080, 3060, 3020, 1600, 1490, 750, 700. EIMS *m/z* (rel. int.): 284 $[M+1]^+$ (2), 266 (1), 176 (100), 158 (20), 133 (12).

*Acetyl deoxyclausenamide* (**1c**). EIMS *m/z* (rel. int.): 368 $[M+1]^+$ (0.3), 308 (1.5), 218 (30), 158 (100).

*Clausenamidone* (**1d**). White prisms, mp 207–210°; IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3260, 3060, 3040, 1690, 1670, 1600, 1500; UV $\lambda_{max}^{MeOH}$ nm (log ε): 252 (4.07). EIMS *m/z* (rel. int.): 295 $[M]^+$ (1), 190 (100), 162 (45), 134 (70), 133 (40), 105 (35), 91 (20), 77 (50).

*Acetyl dehydroxyclausenamide* (**1e**). IR $\nu_{max}^{film}$ $cm^{-1}$: 1740, 1710, 1203; $^1H$ NMR (200 MHz, $CDCl_3$); δ 2.10 (3H, *s*, MeCOO), 2.49 (2H, *dd*, *J* = 2, 8 Hz, H-7), 2.56 (3H, *s*, H-6), 3.78–4.18 (2H, *m*, H-4, H-5), 6.03 (1H, *d*, *J* = 10 Hz, H-3), 6.78–7.00 (2H, *m*, aromatic H), 7.11–7.51 (8H, *m*, aromatic H); EIMS *m/z* (rel. int.): 324 $[M+1]^+$ (37), 264 (4), 232 (39), 172 (100), 91 (39).

*Dehydroxyclausenamide* (**1f**). **1e** was saponified in KOH–MeOH to give **1f** as white granular crystals, mp 123–125°; IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3290, 1680; EIMS *m/z* (rel. int.): 282 $[M+1]^+$ (5), 190 (100), 162 (38), 134 (47), 91 (42).

*Anhydroclausenamide* (**1g**). White needles, mp 198–201°; IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3180, 1695, 1668, 1600, 1500, 770, 700; UV $\lambda_{max}^{MeOH}$ nm (log ε): 215 (4.31), 291 (4.36); EIMS *m/z* (rel. int.): 279 $[M]^+$ (11), 188 (100), 160 (9), 91 (41); HRMS: 279.1189 $[M]^+$ ($C_{18}H_{17}NO_2$), 188.0707 ($C_{11}H_{10}NO_2$), 160.0731 ($C_{10}H_{10}NO$).

*Methyl anhydroclausenamide* (**1h**). **1g** was methylated with $CH_2N_2$ in $Et_2O$ to give **1h**; IR $\nu_{max}^{film}$ $cm^{-1}$: 1690, 1644, 1600, 1500, 760, 700; EIMS *m/z* (rel. int.): 293 $[M]^+$ (10), 202 (100).

*Anhydrocycloclausenamide* (**1i**). White needles mp 188–190°; EIMS *m/z* (rel. int.): 279 $[M]^+$ (100); HRMS: 279. 1176 $[M]^+$ ($C_{18}H_{17}NO_2$); IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3300, 1680, 1600, 1490, 768, 750, 710; UV $\lambda_{max}^{MeOH}$ nm (log ε): 220 (3.60), 263 (2.90).

*Acetyl-anhydrocycloclausenamide* (**1j**). **1i** was acetylated to give **1j** as a white amorphous solid, mp 148–151°; IR $\nu_{max}^{film}$ $cm^{-1}$: 3060, 3030, 1745, 1710, 1600, 1500, 1230, 750, 720, 700; EIMS *m/z* (rel. int.): 321 $[M]^+$ (5), 279 (30), 261 (100).

*Neoclausenamide* (**2**). White cubes, mp 205–206°; Found: C 73.00, H 6.46, N 4.50%, calc. for $C_{18}H_{19}NO_3$: C 72.76, H 6.45, N 4.72%; IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3440, 3340, 3060, 3030, 1660, 1600, 1490, 750, 700; EIMS *m/z* (rel. int.): 298 $[M+1]^+$ (0.4), 191 (38), 190 (100), 174 (14), 162 (38), 144 (1), 134 (60), 133 (38), 119 (22), 107 (18), 105 (18), 91 (30), 77 (38). **HRMS**: 298.1453 $[M+1]^+$ ($C_{18}H_{20}NO_3$).

*Acetyl neoclausenamide* (**2a**). Found: C 68.85, H 5.96, N 3.36%, calc. for $C_{22}H_{23}NO_5$: C 69.29, H 6.04, N 3.6%; IR $\nu_{max}^{film}$ $cm^{-1}$: 3060, 3040, 1745, 1708, 1600, 1495, 1225, 750, 730, 700; EIMS *m/z* (rel. int.): 382 $[M+1]^+$ (0.5), 321 (0.1), 262 (5), 232 (60), 172 (100), 144 (30), 91 (18), 77 (15), 43 (42).

*Neoclausenamidone* (**2b**). Mp 202–205°, IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3280, 3070, 3040, 1690, 1600, 1500, 760, 700; EIMS *m/z* (rel. int.): 295 $[M]^+$ (0.5), 190 (100), 162 (30), 134 (45), 133 (20), 105 (30), 91 (18), 77 (40).

*Cycloclausenamide* (**3**). White prisms, mp 164–166°; Found: C 77.06, H 6.13, N 4.76%, calc. for $C_{18}H_{17}NO_2$: C 77.42, H 6.09, N 5.02%.

*Acknowledgement*—The authors gratefully acknowledge Mr David Noble for his help in revising the paper.

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