The Structure of Roxburghilin, a Bis-amide of 2-Aminopyrrolidine from the Leaves of *Aglaia roxburghiana* (Meliaceae)

By **Kozhiparambil K. Purushothaman** and **Ayyapath Sarada**, Captain Srinivasa Murti Research Institute, Madras, India
Joseph D. Connolly * and **John A. Akinniyi**, Chemistry Department, University of Glasgow, Glasgow G12 8QQ, Scotland

Roxburghilin, a bis-amide from the leaves of *Aglaia roxburghiana* (Meliaceae) has been shown to be *N*-cinnamoyl-2-(2-methylbutanoylamino)pyrrolidine (1) by a combination of chemical and spectroscopic evidence, and by synthesis of the corresponding dihydro-derivative (4) from L-proline.

FROM the leaves of *Aglaia roxburghiana* (Meliaceae) we have isolated a new bis-amide, roxburghilin, which has been shown to be *N*-cinnamoyl-2-(2-methylbutanoylamino)pyrrolidine (1).

RESULTS AND DISCUSSION

Roxburghilin (1), $C_{18}H_{24}O_2N_2$ $[\alpha]_D$ +34°, had bands in the i.r. at 3 442, 3 280 (NH), 1 686, and 1 660 (amide) cm^{-1}. The ^1H n.m.r. spectrum indicated the presence of cinnamoyl [δ 6.94 and 7.64 (AB q, *J* 16 Hz) and *ca.* 7.4 (5 H, m)] and 2-methylbutanoyl [δ 0.76 (t, *J* 7 Hz, CH_2Me), 1.12 (d, *J* 7 Hz, $CHMe$), and 1.49 (2 H, m, CH_2Me)] residues and one NH [δ 6.60, d, *J* 8 Hz; disappeared on addition of D_2O—CF_3CO_2H]. Alkaline hydrolysis of (1) afforded cinnamic acid and (+)-2-methylbutanoic acid. The latter was characterised as its *p*-bromoanilide. The two acid residues and the remaining carbons were also readily identified in the ^{13}C n.m.r. spectrum of (1) (see Table 1). Thus roxburghilin was a secondary-tertiary bis-amide of a monocyclic unit $C_4H_8N_2$ comprising two methylenes, a methylene bearing nitrogen, and a methine bearing two nitrogens. The corresponding ^1H resonances appeared at δ 2 (4 H, m), 3.62 [2 H, m, CH_2–N] and 6.12 [1 H, m, N–CH–N]. Irradiation at δ 2, the methylene resonance, resulted in the simultaneous collapse of the CH_2–N multiplet to an AB quartet [δ 3.48 and 3.74, *J* 12 Hz] and the N–CH–N signal to a doublet (*J* 8 Hz), coupled with the NH doublet at δ 6.60. These results were satisfactorily interpreted in terms of a 2-aminopyrrolidine nucleus and led to structures (1) or (2) for roxburghilin.

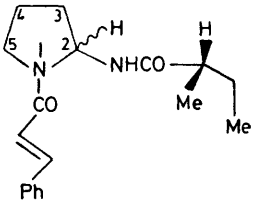
TABLE 1

^{13}C N.m.r. spectra of roxburghilin and related compounds (in $CDCl_3$ at room temperature)

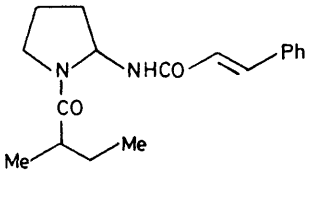
	(1)	(4) [a]	(4) [b]	(12) [c]	(11) [c]
C-2	62.8, 62.7 [d]	63.5 (65.0)	64.6	64.3	66.2
C-3	34.5	34.4 (36.7)	34.0 [d]	34.6	34.6
C-4	21.6 [d]	21.5 (23.8)	22.4 [d]	21.6	21.4
C-5	46.2	47.2 (45.7)	46.5	45.8	45.5
Tertiary amide	165.7 (s)	172.1	171.8	170.4	170.5
	142.8 (d)	35.6	36.2	22.0	22.0
	118.2 (d)	31.3, 31.0 [d]	31.7		
	134.8 (s)	140.9	142.0		
	129.9 (d)	126.1	126.0		
	128.8(2) (d)	128.4(4)	128.9(2)		
	128.3(2) (d)		128.7(2)		
					(urethane)
Secondary amide	175.9(s)	175.6	175.4	175.6	155.3
	42.9, 42.7(d) [d]	42.9 [d]	43.0	42.8	61.0
	27.3, 27.0(t)	27.2, 27.0 [d]	27.4, 27.7 [d]	27.0	14.6
	17.6, 17.3(q) [d]	17.6, 17.3 [d]	17.5, 17.4 [d]	17.4	
	11.9(q)	11.9	11.9	11.9	

[a] Figures in parentheses represent minor rotamer. [b] $[^2H_8]$-Toluene at 100 °C. [c] Major rotamer. [d] Presence of epimers indicated by two resonances or unresolved shoulder.

The mass spectrum of roxburghilin had strong peaks at *m/e* 169 and 131, and 215 and 85, resulting from loss of



(1)

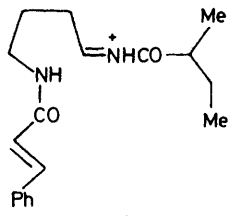


(2)

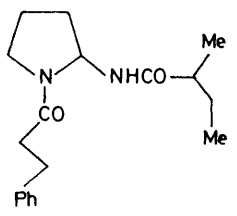
the cinnamoyl and 2-methylbutanoyl fragments. In addition there was a peak at *m/e* 199 [M^+, 199.099 68. $C_{13}H_{13}NO$ requires *M*, 199.099 70] corresponding to loss of 2-methylbutanoic acid amide, presumably by a McLafferty rearrangement. This result indicated that the 2-methylbutanoic acid was associated with the secondary amide function and confirmed the structure of roxburghilin as *N*-cinnamoyl-2-(2-methylbutanoylamino]-pyrrolidine (1). The configuration at C-2 was not determined. The isolation of (+)-2-methylbutanoic acid on hydrolysis of (1) indicated that the configuration at C-2′ is (*S*).[1] The biogenetic origin of roxburghilin is not known, but it may be derived from ornithine *via* an acylated putrescine intermediate.

On standing in chloroform solution roxburghilin underwent partial epimerisation at C-2. This was apparent in both the ^1H n.m.r. spectrum, which showed two sets of methyl signals, and in the ^{13}C n.m.r. spectrum which exhibited doubling of some of the resonances (see Table 1). In addition two spots were observed on analytical t.l.c. The equilibrium was accelerated by addition of a drop of trifluoroacetic acid, and presumably proceeds *via* a ring-opened intermediate, *e.g.* (3).

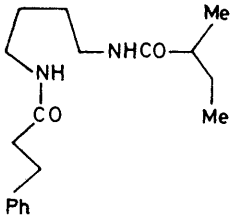
Careful preparative t.l.c. of the equilibrium mixture resulted in the isolation of 2-epiroxburghilin which had the same ^{13}C n.m.r. spectrum as roxburghilin, as a result of equilibration at C-2 during spectrum accumulation.



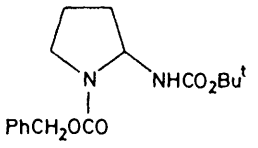
(3)



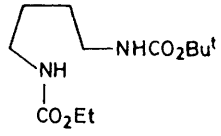
(4)



(5)



(6)



(7)

Depending on the conditions, hydrogenation of roxburghilin yielded not only dihydroroxburghilin (4) but also the ring-cleaved product tetrahydroroxburghilin (5). Reductive cleavage of a similar system (6), with sodium borohydride, to give compound (7), has been reported recently.[2] Treatment with acid caused equilibration * at C-2 in dihydroroxburghilin (4) but the epimers had identical ^1H n.m.r. spectra and chromatographic properties and could not be separated. Hydrogenation of an equilibrium mixture of roxburghilin and 2-epiroxburghilin afforded a single dihydro-product, indistinguishable from dihydroroxburghilin (4) (t.l.c. and ^1H n.m.r.).

The ^{13}C n.m.r. spectrum of dihydroroxburghilin (4), unlike that of roxburghilin, exhibited two sets of signals for the pyrrolidine ring carbons due to restricted rotation about the tertiary amide. As expected only one set of signals was observed at 100° in [$^2H_8$]toluene. It is interesting to note that in dihydroroxburghilin and the simple amides, *N*-cinnamoyl- and *N*-dihydrocinnamoylpyrrolidine, the chemical shift difference between C-3 and C-4 of the rotamers is greater than that between C-2 and C-5. The corresponding piperidine derivatives show the opposite effect (see Table 2).

The 2-aminopyrrolidine system has been synthesised [3]

* The presence of the epimers was detected by ^{13}C n.m.r. (see Table 1).

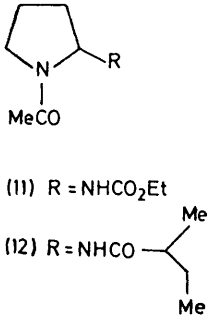
TABLE 2

^{13}C N.m.r. chemical-shift differences of ring carbons in amide rotamers

Compound	C-2, C-5	Δδ	C-3, C-4	Δδ
Dihydroroxburghilin (4) [a]	64.6, 63.8	0.8	36.9, 34.8	2.1
	46.9, 46.1	0.8	23.8, 21.6	2.2
N-Dihydrocinnamoylpyrrolidine [a]	46.0, 45.8	0.2	26.1, 24.4	1.7
N-Cinnamoylpyrrolidine [a]	46.0	0	26.2, 24.4	1.8
	C-2, C-6		C-3, C-5	
N-Dihydrocinnamoylpiperidine [b]	47.0, 43.3	3.7	26.7, 25.7	1.0
N-Cinnamoylpiperidine [b]	46.6, 42.7	3.9	26.4, 25.5	0.9

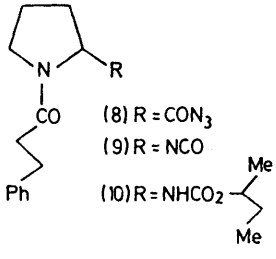
[a] [$^2H_8$]Toluene. [b] In $CDCl_3$. The Δδ value for *NN*-dimethylacetamide in $CDCl_3$ is 3.0 p.p.m.

but roxburghilin appears to be the first example isolated from a natural source. We decided to confirm the structure by synthesis of dihydroroxburghilin (4). Curtius rearrangement of the azide (8) of *N*-dihydrocinnamoyl-L-proline afforded the optically active isocyanate (9) which was reacted with 2-butylmagnesium bromide to give the bis-amide (4), with the same chromatographic and spectroscopic properties as dihydroroxburghilin. It is probable that the synthetic material is totally racemic as a result of equilibration at C-2 during the acidic work-up of the Grignard reaction. Another product of the reaction was the urethane (10). The butan-2-ol required for the formation of (10) presumably arose by reaction of the Grignard reagent with oxygen. In the course of this work the model compounds (11) and (12) were also prepared. Their ^{13}C data appear in Table 1.



(11) R = $NHCO_2Et$

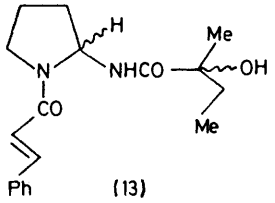
(12) R = NHCO



(8) R = CON_3

(9) R = NCO

(10) R = $NHCO_2$



(13)

A second bis-amide, $C_{18}H_{24}N_2O_3$ [*m/e* 316; $\nu_{max.}$ (CCl_4) 3 622, 3 420, 3 300, 1 680, and 1 660 cm^{-1}], isolated from the extract in minor amount, has been assigned structure (13). Its spectroscopic properties were similar to those of roxburghilin (see Experimental section). The obvious differences in the ^1H n.m.r. spectra were the re-

placement of the secondary methyl signal of (1) by a methyl singlet at δ 1.33 and the appearance of a tertiary hydroxy proton (exchangeable with D_2O) at δ 2.48. The above data clarly indicated the presence of a 2-hydroxy-2-methylbutanoyl residue.

EXPERIMENTAL

For general experimental details see ref. 4.

Extraction.—Coarsely powdered leaves of *Aglaia roxburghiana* were successively extracted with hexane and chloroform in the cold. The hexane extract contained β-sitosterol and several tetracyclic triterpenoids. The chloroform extract was chromatographed over Grade IV alumina in benzene. The fractions eluted with benzene were combined and crystallised from ethyl acetate to give *roxburghilin* (1), m.p. 205 °C (Found: C, 71.8; H, 8.2; N, 9.2. $C_{18}H_{24}O_2N_2$ requires C, 72.0; H, 8.0; N, 9.3%). Preparative t.l.c. of the mother-liquors afforded a small quantity of *compound* (13) [δ 0.90 (t, *J* 7 Hz, CH_2Me), 2.0 (4 H, m, ring methylenes), 3.62 (2 H, m, CH_2–N), 6.12 (1 H, m, N–CH–N), 6.94 and 7.66 (AB q, *J* 16 Hz, Ph–CH=CH–CO), and *ca.* 7.4 (5 H, m, phenyl)] (Found: M^+ 316.178 49. $C_{18}H_{24}O_3N_2$ requires *M*, 316.178 67).

Hydrolysis of Roxburghilin.—(*a*) Roxburghilin (300 mg) was refluxed for 6 h in 5% ethanolic potassium hydroxide solution (15 ml). The ethanol was removed *in vacuo* and the residue acidified with 5M hydrochloric acid, extracted with dichloromethane and the crude product was chromatographed over silica gel. The fractions eluted with benzene yielded a solid which was crystallised from water to give cinnamic acid, m.p. 133 °C, identical with an authentic specimen.

(*b*) Roxburghilin (1 g) was hydrolysed as above. The dichloromethane extract was distilled off and the fraction boiling at 180 °C was redistilled to give 2-methylbutanoic acid, b.p. 177 °C. This was characterised as its *p*-bromoanilide, prepared in the usual way and recrystallised from ether–hexane, m.p. 132—134 °C; $[\alpha]_D$ +32° (*c* 0.08); *m/e* 257 and 255, identical with an authentic specimen [lit.,[1] m.p. 132—134 °C; $[\alpha]_D$ +32° (acetone)].

Hydrogenation of Roxburghilin.—Roxburghilin (37 mg) in ethyl acetate (20 ml) was hydrogenated over 10% Pd–C for 5 min. The catalyst was filtered off and the solvent removed to give a quantitative yield of *dihydroroxburghilin* (4), which was crystallised from chloroform–ether, m.p. 105—106 °C; $[\alpha]_D$ 0°; *m/e* 302; $\nu_{max.}$ (CCl_4) 3 442, 3 300, 1 685, and 1 662 cm^{-1}; δ_H 0.78 (3 H, t, *J* 7 Hz, $MeCH_2$), 1.02 (3 H, d, *J* 7 Hz, *Me*CH), *ca.* 2.6 and 2.95 (each 2 H, m, $PhCH_2CH_2CO$), 3.45 (2 H, m, CH_2–N), 5.65 (1 H, m, N–CH–NH), 6.06 (1 H, m, NH), and 7.18 (5 H, br s, phenyl) (Found: C, 71.5; H, 8.8; N, 9.3. $C_{18}H_{26}O_2N_2$ requires C, 71.5; H, 8.7; N, 9.3%). Prolonged hydrogenation under the above conditions afforded *tetrahydroroxburghilin* (5) which was crystallised from ethyl acetate–ether, m.p. 133—137 °C; $\nu_{max.}$ (CCl_4) 3 560, 3 325, 1 681, and 1 672(sh) cm^{-1}; δ_H 0.91 (3 H, t, *J* 7 Hz, $MeCH_2$), 1.15 (3 H, d, *J* 7 Hz, *Me*CH), 2.47 and 2.96 (each 2 H, m, $PhCH_2CH_2CO$), 3.2 (4 H, m, 2 × CH_2N), 5.95 (2 H, m, 2 × N*H*, exchangeable with D_2O–CF_3CO_2H), and 6.19 (5 H, m, phenyl) (Found: M^+, 304.214 82. $C_{18}H_{28}O_2N_2$ requires *M*, 304.215 07). The amount of tetrahydro-compound formed varied with the batch of catalyst used. In some experiments the yield was very low even after overnight reaction.

Equilibration of Roxburghilin.—Roxburghilin (20 mg) in chloroform solution was stirred with trifluoroacetic acid (2 drops) in water (5 drops) for a few minutes. Analytical t.l.c. of the product showed two spots of similar R_F values. Careful repetitive preparative t.l.c. afforded two compounds. The more polar was roxburghilin. The less polar was 2-epiroxburghilin (7 mg) which was recrystallised from chloroform–light petroleum, m.p. 171—172 °C; δ_H 0.9 (3 H, t, *J* 7 Hz, $MeCH_2$), 1.06 (3 H, d, *J* 7 Hz, *Me*CH), *ca.* 1.97 (4 H, m, CH_2CH_2), 3.54 (2 H, m, CH_2N), 6.14 (1 H, m, N–C*H*–NH), 6.86 (1 H, br d, *J* 8 Hz, NH), 6.92 and 7.68 (AB q, *J* 16 Hz, PhCH=CHCO), and *ca.* 7.4 (5 H, m, phenyl) (Found: M^+ 300. $C_{18}H_{24}O_2N_2$ requires *M*, 300). Hydrogenation of the equilibrium mixture afforded a product identical with dihydroroxburghilin (4) (t.l.c. and ^1H n.m.r.).

Synthesis of Dihydroroxburghilin (4).—L-Proline (8.7 g) was dissolved in 7M sodium hydroxide solution (20 ml) and dihydrocinnamoyl chloride (12.8 g) added with stirring. After 5 min the mixture was acidified and left overnight in the refrigerator. The precipitated N-*dihydrocinnamoyl*-L-*proline* (8 g) was filtered off and crystallised from chloroform–light petroleum, m.p. 98—100 °C; $[\alpha]_D$ −111° (water); δ_H 2.1 (4 H, m, CH_2–CH_2), 2.65 and 3.0 (each 2 H, m, $PhCH_2CH_2CO$), 3.45 (2 H, m, 5-H_2), 4.58 (1 H, m, H-2), 7.25 (5 H, br s, phenyl), and 10.3 (1 H, s, CO_2H). The corresponding *methyl ester*, prepared in the usual way by reaction with diazomethane in methanol, was obtained as an oil, *m/e* 261; $\nu_{max.}$ (thin film) 1 737 and 1 640 cm^{-1}; δ_H 2.0 (4 H, m, CH_2CH_2), 2.58 and 3.0 (each 2 H, m, $PhCH_2CH_2CO$), 3.51 (2 H, m, H_2-5), 3.71 (3 H, s, CO_2Me), 4.48 (1 H, m, H-2), and 7.3 (5 H, br s, phenyl). The methyl ester (6.0 g), in ethanol solution, was allowed to stand overnight at room temperature with an excess of hydrazine hydrate. The hydrazide (5.4 g), obtained on removal of the solvent *in vacuo*, was suspended in a mixture of concentrated hydrochloric acid–acetic acid (10 : 1) and cooled in a salt–ice bath. An aqueous solution of sodium nitrite was added dropwise. The reaction mixture was extracted with ethanol-free chloroform and the organic layer dried over anhydrous sodium sulphate–sodium carbonate. The formation of the azide (8) (5 g) was confirmed by the presence of a band in the i.r. spectrum at 2 130 cm^{-1}. Conversion of the azide into the isocyanate (9) was achieved by warming the chloroform solution at 50—60 °C in an oil-bath for 2.5 h. The reaction was monitored by the appearance of the isocyanate band in the i.r. at 2 260 cm^{-1}, and the disappearance of the azide band at 2 130 cm^{-1}. The isocyanate (9) (4.8 g), in dry tetrahydrofuran, was treated with 2-butylmagnesium bromide in a nitrogen atmosphere and refluxed overnight. The reaction was worked-up by addition of saturated aqueous ammonium chloride solution and extraction with chloroform. The crude product was chromatographed over Grade IV alumina. Elution with 30% chloroform–light petroleum afforded dihydroroxburghilin (4) (500 mg), which was purified by preparative t.l.c. and crystallised from chloroform–light petroleum as needles, m.p. 108—109 °C; $[\alpha]_D$ 0°. The spectroscopic properties of the synthetic product were identical with those of the naturally derived compound. A less polar product from the column, eluted with 20% chloroform–light petroleum, was the non-crystalline *urethane* (10) (200 mg); *m/e* 318; $\nu_{max.}$ (CCl_4) 3 450, 3 300, 1 664, and 1 724 cm^{-1}; δ 0.87 (3 H, t, *J* 7 Hz, $MeCH_2$), 1.2 (3 H, d, *J* 7 Hz, *Me*CH), 3.5 (2 H, m, H_2-5), 4.8 (1 H, m, CH–O), 5.16 (1 H, m, NH), and 5.53 (1 H, m, H-2).

Model Compounds (11) *and* (12).—*N*-Acetyl-L-proline was subjected to Curtius rearrangement as above. The result-

ing isocyanate was reacted with ethanol to give the urethane (11); *m/e* 228; $\nu_{max.}$ (CCl_4) 3 440, 3 282, 1 725, and 1 660 cm^{-1}; δ_H (at 65 °C in $CDCl_3$) 1.20 (3 H, t, *J* 7 Hz, *Me*CH_2), 2.07 (3 H, s, MeCO), 3.45 (2 H, m, H_2-5), 4.11 (2 H, q, *J* 7 Hz, MeCH_2O), and 5.5 (2 H, m, H-2 and NH). In a separate experiment the isocyanate was treated with 2-butylmagnesium bromide to yield the *bis-amide* (12), which was crystallised from chloroform–light petroleum, m.p. 118—120 °C; *m/e* 212; $\nu_{max.}$ (CCl_4) 3 445, 3 300, 1 680, and 1 664 cm^{-1}; δ_H 0.90 (3 H, t, *J* 7 Hz, *Me*CH_2), 1.13 (3 H, d, *J* 7 Hz, *Me*CH), 2.08 (3 H, s, MeCO), 3.47 (2 H, m, H_2-5), 5.85 (1 H, m, H-2), and 6.93 (1 H, br d, *J* 8 Hz, NH). (Found: C, 62.05; H, 9.25; N, 13.15. $C_{11}H_{20}N_2O_2$ requires C, 62.25; H, 9.5; N, 13.2%).

We acknowledge financial support from the Central Council for Research in Indian Medicine and Homeopathy, New Delhi (K. K. P. and A. S.), the University of Maiduguri, Nigeria (J. A. A.) and an equipment grant from the S.R.C. (J. D. C.).

[9/209 *Received*, 12*th February*, 1979]

REFERENCES

[1] (*a*) T. Nakano, C. Djerassi, R. A. Corral, and O. O. Orazi, *J. Org. Chem.*, 1961, **26**, 1184; (*b*) A. Kjaer and S. E. Hansen, *Acta Chem. Scand.*, 1957, **11**, 898.

[2] K. Murato, T. Shioiri, and S. Yamada, *Chem. Pharm. Bull.* (*Japan*), 1977, **25**, 1559.

[3] K. Murato, T. Shioiri, and S. Yamada, *Chem. Pharm. Bull.* (*Japan*), 1975, **23**, 1738.

[4] S. F. Kimbu, T. K. Njimi, B. L. Sondengam, J. A. Akinniyi, and J. D. Connolly, *J.C.S. Perkin I*, 1979, 1303.