A Conformational Study of Diterpenoid Lactones Isolated from the Chinese Medicinal Herb *Andrographis paniculata*

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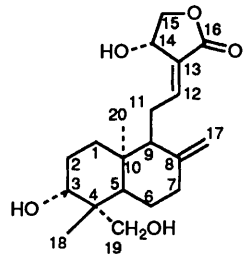
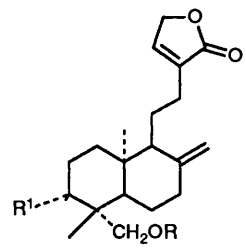
X-Ray crystallography and 500 MHz proton NMR spectroscopy have been used to investigate the conformations of four diterpenoid lactones isolated from extracts of the shrub *Andrographis paniculata*. The crystal structure of neoandrographolide (**2**) was refined using a blocked-diagonal least squares technique with 340 parameters and 2 018 reflections to yield an *R* value of 0.049. Full-matrix least-squares refinement for 14-deoxyandrographolide (**3**) using 102 parameters and 893 reflections yielded an *R* value of 0.083. The proton NMR spectra of andrographolide (**1**), neoandrographolide, 14-deoxyandrographolide, and 14-deoxy-11,12-didehydroandrographolide (**4**) have been assigned and the conformations of the lactone and glucose side-chains deduced from proton coupling constants.

Extracts of the shrub *Andrographis paniculata* are widely used as herbal medicines in the West Indies, India, and China.[1,2] Diterpenoid lactones isolated from these extracts have been the subject of intensive investigation,[1-3] and the major diterpenoid lactone is andrographolide (**1**). The structure and stereochemistry of andrographolide have been determined over many years using chemical and spectroscopic techniques,[3a] and recently, the crystal structure of andrographolide was determined.[3b] Several minor diterpenoid lactones have also been isolated from *A. paniculata*.[1,3c,3d] Neoandrographolide was first investigated by Kleipool[3c] in 1952; structure (**2**) was later proposed on the basis of chemical and spectroscopic evidence.[1] Balmain and Connolly[3d] have described the isolation and characterisation of 14-deoxyandrographolide (**3**), 14-deoxy-11,12-didehydroandrographolide (**4**) and 14-deoxy-11-oxoandrographolide (**5**). Chen and Liang[2] have also isolated deoxyandrographolide-19β-D-glucoside (**6**) from the leaves of *A. paniculata*.

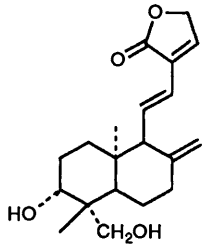
Comparatively little is known about the solution conformation of these diterpenoid lactones. Only with the development of very high-field spectrometers and two-dimensional NMR techniques[4] has it become possible to completely assign the proton NMR spectra of such molecules. Recent work in our laboratories has involved the isolation of diterpenoid lactones from *A. paniculata* in connection with a virological project. It therefore seemed appropriate to reinvestigate these compounds with the aim of adding definitively, to the literature already available,[1-3] further structural data from a high-field (500 MHz) proton NMR study and additional X-ray diffraction studies.



(1)



(2) R = glucose, R^1 = H
(3) R = H, R^1 = OH
(6) R = glucose, R^1 = OH



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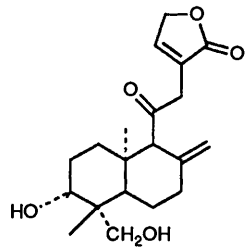

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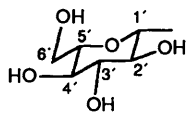

Results and Discussion

Extraction of the whole plant (Experimental section) gave andrographolide (**1**) and neoandrographolide (**2**) as colourless needles. The crystal structure of andrographolide has been determined by Smith and co-workers,[3b] but none of the minor constituents have been the subject of an X-ray diffraction study. Crystals of neoandrographolide (**2**) were therefore obtained by slow evaporation of an acetone solution and used for a structure determination. The structure agrees with that proposed previously[1] (Figure 1). Neoandrographolide crystallises as a monoclinic system with $P2_1$ space group. The unit cell consists of two molecules of neoandrographolide with a = 7.351(2) Å, b = 6.207(1) Å, c = 28.456(6) Å, and β = 95.22(2)°. Details of the structure determination and crystal data are given in the Experimental section and in Table 1.

A particularly interesting feature of the structure of neoandrographolide is the water of crystallization which hydrogen bonds

Table 1. Data collection and structure refinement information for neoandrographolide (**2**) and 14-deoxyandrographolide (**3**).

	Neoandrographolide	14-Deoxyandrographolide
Formula	$C_{26}H_{42}O_9$	$C_{22}H_{30}O_4$
M_w	498.62	334.46
Colour and habit	Colourless needles	Colourless needles
Dimensions/mm	0.025 × 0.03 × 0.40	0.025 × 0.17 × 0.26
Calc. density/g cm^{-3}	1.28	1.25
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1$	$P2_1$
a/Å	7.351(2)	6.701(2)
b/Å	6.207(1)	6.862(4)
c/Å	28.456(6)	19.282(6)
β/°	95.22(2)	92.41(3)
V/Å^3	1 293.1(5)	885.9(7)
T/°	130 K	130 K
Z	2	2
λ	Mo-K_α, 0.710 69 Å	Mo-K_α, 0.710 69 Å
μMo-K_α/cm^{-1}	0.89	0.80
Range of trans. factors	0.99–1.00	0.99–1.00
Scan method	w, 1.0° range 1.0° offset for background	w, 1.5° range 1.2° offset for background
Scan speed/° min^{-1}	6	4
2θ range/°	0–50	0–45
Octants collected	$h, k, +/-1$	$h, k, +/-1$
Data collected	2 712	1 399
Unique data	2 511	1 281
Data refined [$I > 2\sigma(I)$]	2 018	893
Parameters refined	340	102
R	0.049	0.083
R_w	0.047	0.082
w	$[\sigma^2(F_o) + 0.000\,83F_o^2]^{-1}$	$[\sigma^2(F_o) + 0.001\,37F_o^2]^{-1}$

$R = S||F_o| - |F_c||/|F^o|$ and $Rw = S||F_o| - |F_c||w^{\frac{1}{2}}/|F_o w^{\frac{1}{2}}|$.

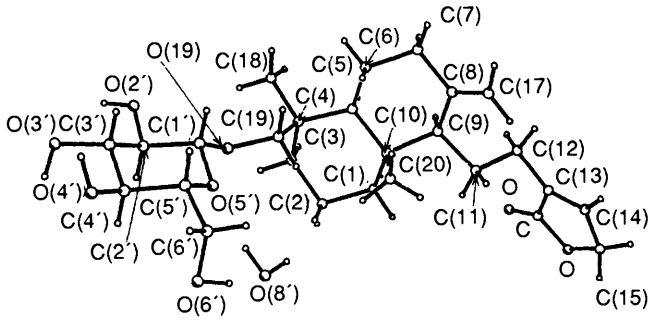

Figure 1. Projection from the crystal structure of neoandrographolide (**2**).

Table 2. Hydrogen-bond distances/Å and angles/° for neoandrographolide (**2**). O(8′) is in the water of crystallisation.

Atoms	O–O	O–H–O	H···O
O(2′)–H···O(3′)	2.80	153	2.02
O(3′)–H···O(6′)	2.64	169	1.70
O(4′)–H···O(3′)	2.86	173	1.98
O(6′)–H···O(8′)	2.76	160	1.77
O(8′)–H···O(7′)	2.82	129	2.08

to O(6′) and O(5′) of the glucose ring in a cyclic arrangement (Figure 1). The principle of maximum hydrogen bonding[5] is complied with by virtue of a group of intermolecular hydrogen bonds (Table 2) from OH(2′) and OH(3′) to O(3′) and O(6′) of a second molecule, and OH(4′) to O(3′) of a third molecule. The C–C and C–O bond lengths and angles are similar to those reported for andrographolide.[3b] The sp^3–sp^3 C–C bond lengths vary between 1.51 and 1.58 Å, whereas the sp^3–sp^2 bond lengths are between 1.47 and 1.52 Å. Both sp^2–sp^2 carbon bonds are 1.31 Å and the sp^3–sp^3 carbon–oxygen bonds fall between 1.37 and 1.45 Å.

The side-chain conformations in the crystal structure are those which are expected to minimise steric interactions with the *trans*-decalin moiety, *e.g.* O(19) is in a *trans* arrangement with C(5), whereas the C(1′)–O(19)–C(19)–C(4) unit is close to a *trans* conformation (dihedral angle 155°). A similar situation pertains for the C(10)–C(9)–C(11)–C(12) and C(9)–C(11)–C(12)–C(13) units of the lactone side chain, where the dihedral angles are 165 and 172°, respectively. The lactone ring is almost perpendicular to the C(9)–C(11)–C(12)–C(13) plane. The crystal structure of andrographolide[3b] shows a similar arrangement of the O(19)–C(19)–C(4)–C(5), C(10)–C(9)–C(11)–C(12), and C(9)–C(11)–C(12)–C(13) bonds. Intriguingly, the lactone C=C–C=O fragment in andrographolide is not planar and has a dihedral angle of 11.3°. The lactone ring was described as an envelope with C(15) approximately 0.30 Å out of the mean plane. In contrast, the lactone ring in neoandrographolide (**2**) is essentially planar.

To allow a comparison of the lactone side-chain conformations of these molecules in the crystal structure and in solution the 500 MHz proton NMR spectra of andrographolide and neoandrographolide were assigned. Vicinal proton coupling constants were then used to determine the conformation of the side-chains. The only previous study of the NMR spectrum of neoandrographolide is due to Cava *et al.*,[3a] who were only able to assign the two methyl signals and the downfield signal for H(14). Unfortunately, the authors neglected to note the conditions used or the field strength of their spectrometer. We began our assignment of the 500 MHz spectrum of neoandrographolide (**2**) with solubility tests to determine an appropriate solvent. [^{2}H]$_4$methanol was finally chosen because it gave the

best dispersion and also deuteriated the hydroxy groups. (For a Figure of the spectrum, see supplementary material*). The assignments were then achieved using a combination of decoupling experiments, homonuclear chemical shift correlation and NOE difference spectroscopy. The spectrum in $[^2H]_6$acetone and a benzene titration in $[^2H]_4$methanol were also valuable, allowing the separation of all multiplets except two which overlapped at 1.6–1.7 ppm.

A suitable entry point to begin the assignment was the downfield signal for H(14); decoupling H(14) removed a coupling of 1.7 Hz to the signal at 4.82 ppm (integral two protons) which was assigned as H(15a) and H(15b). The broad singlets at δ 4.86 and 4.63 were therefore H(17a) and H(17b) of the exocyclic vinyl group (Ha of a geminal pair is defined as the downfield proton). Protons in the 4.2 to 3.1 ppm region arise from the methylene at C(19) and the attached glucose. Decoupling experiments established connectivities between H(1′) (4.17 ppm) and H(2′) (3.16 ppm), H(19a) (4.09 ppm) and H(19b) (3.22 ppm), H(6′a) (3.85 ppm) and H(6′b) (3.68 ppm), H(6′b) and H(5′) (3.23 ppm) and H(2′) and H(3′) [H(3′) overlaps with H(4′) and the methanol peak at 3.31 ppm]. The glucose protons for deoxyandrographolide-19β-D-glucoside penta-acetate[4] have been assigned previously (400 MHz; $CDCl_3$) and have very different chemical shifts [H(1′) 4.45, H(2′) 5.05, H(3′) 5.23, H(4′) 5.13, H(5′) not assigned, H(6′a) and H(6′b) 4.34 and 4.17 ppm].

Of some interest are the coupling constants between H(5′) and H(6′a) (2.3 Hz) and H(5′) and H(6′b) (5.4 Hz). Similar coupling constants are also observed for this fragment in deoxyandrographolide-19β-D-glucoside penta-acetate[2] in $CDCl_3$. If the *gauche–gauche* (*gg*) conformation observed at C(5)–C(6) in the crystal structure were the only conformation present in solution, these coupling constants would be equivalent. This is clearly not the case, so the *trans–gauche* (*tg*) or *gauche–trans* (*gt*) conformations must also be populated in solution. Nishida *et al.*[6] have shown by chiral deuteriation at the C-6 position of D-glucoses that there is a negligibly low population of the *tg* conformation and that the equilibrium is approximately 60% *gg* and 40% *gt*. This ratio is essentially independent of the solvent and protecting group. The observed coupling constants for the glucose ring of neoandrographolide therefore confirm that the presence of the *trans*-decalin moiety also has little effect on the conformation at the C(5′)–C(6′) bond.

At this stage in the assignment a COSY-45 experiment encompassing the region 0.6 to 2.6 ppm was used to assign the ring protons (a combination of decoupling experiments and a COSY-45 experiment over a limited spectral window was found to be much less time consuming than a COSY experiment over the full spectral window; a COSY-45 experiment was chosen to differentiate vicinal and geminal coupling constants. (For a Figure of this spectrum, see supplementary material.*) Starting with the low-field side of the multiplet at 2.4 ppm, a five-spin system can be traced out and assigned as $H(7)_{eq}$ (2.42 ppm), $H(7)_{ax}$ (1.97 ppm), $H(6)_{ax}$ (1.39 ppm), $H(6)_{eq}$ (1.87 ppm), and $H(5)_{ax}$ (1.26 ppm) on the basis of multiplet structures. Beginning with the axial proton at 0.96 ppm, another spin system can also be assigned, with cross peaks to a geminal partner (1.96 ppm) and vicinal protons at 1.63 and 1.46 ppm. Both of these vicinal protons show cross peaks to the axial proton at 1.08 ppm, whose geminal partner is at 1.80 ppm. A long range coupling also connects the equatorial protons at δ 1.96 and 1.80 ppm. A NOE difference experiment with irradiation of the high-field methyl group gave NOEs at 1.63 ppm, 1.80 ppm, H(19a), and H(6a). This pattern of NOEs can only be explained if the methyl being irradiated is Me(20), which is in an axial orientation. The enhancements observed are to all the protons in a 1,3-diaxial orientation with Me(20) [*i.e.* $H(2)_{ax}$, $H(6)_{ax}$, and H(19a)] plus the vicinal equatorial proton $H(1)_{eq}$. This allows a complete assignment of the six-spin system as $H(1)_{ax}$ (1.08 ppm), $H(1)_{eq}$ (1.80 ppm), $H(2)_{ax}$ (1.63 ppm), $H(2)_{eq}$ (1.46 ppm), $H(3)_{ax}$ (0.96), and $H(3)_{eq}$ (1.96 ppm). The enhancement to H(19a) suggests that the solution conformation about the C(19)–C(4) bond is the same as in the crystal structure, with H(19a) in close proximity to Me(20).

The remaining assignments gave information about the conformation of the lactone side chain. The multiplet at 2.40 ppm and a geminal partner at 2.10 ppm were assigned as H(12a) and H(12b) because the only cross-peaks observed were to the vicinal neighbours H(11a) (1.79 ppm) and H(11b) (1.63 ppm). The doublet at 1.68 ppm is therefore $H(9)_{ax}$ (J = 11.0 and <3 Hz). In $[^2H]_6$acetone $H(9)_{ax}$ is well separated from H(11b) and yields similar coupling constants. These coupling constants are consistent with the arrangement of the C(10)–C(9)–C(11)–C(12) unit shown in the crystal structure, in which $H(9)_{ax}$ has a diehedral angle of 180° with H(11a) or H(11b). Decoupling experiments also gave coupling constants of 7.3 Hz [H(12b)–H(11b)] and 6.3 Hz [H(12b)–H(11a)]. Coupling constants to H(12a) could not be reliably measured because of overlap with $H(7)_{eq}$. However, even without further experiments (*e.g.* a homonuclear *J*-resolved spectrum) it is clear that this portion of the side-chain is sufficiently flexible to occupy a *gauche* conformation at the C(11)–C(12) bond in addition to the *trans* conformation observed in the crystal structure. Molecular models show that the (−) *gauche* conformation is sterically crowded, so the vicinal coupling coupling constants for H(12b) are best interpreted in terms of an appreciable population of the (+) *gauche* conformer. To complete the assignments, NOE difference experiments with irradiation of H(17b) and H(17a) were carried out. These showed that H(17b) is the proton pointing towards H(12a).

On the basis of these results, the conformational equilibrium of neoandrographolide (**2**) in solution is significantly different from that suggested by the crystal structure. In particular, the *gt* conformation at the C(5′)–C(6′) bond is populated in addition to the *gg* conformation seen in the crystal structure. Furthermore, coupling constants for the C(11)–C(12) unit indicate that both the *trans* and (+) *gauche* conformers are populated in solution, whereas in the crystal structure only a *trans* conformation is observed.

The 500 MHz NMR spectrum of andrographolide (**1**) was obtained using the same conditions. (For a Figure of the spectrum, see supplementary material.*) Several areas of the spectrum overlap quite badly, but a complete assignment was possible. The downfield signal for H(12) was used as a starting point. A decoupling experiment established couplings of 6.8 Hz to the protons at 2.63 ppm [H(11a)] and 2.58 ppm [H(11b)]. Although these protons overlap somewhat, the vicinal couplings to H(9) (1.92 ppm) of 3.9 Hz for H(11a) and 10.2 Hz for H(11b) can be obtained from a first-order analysis. The large geminal coupling for H(11a) and H(11b) (16.5 Hz) has been observed previously for similar systems and was attributed to the hyperconjugative effect of the vinyl group.[7]

Turning now to the conformation at the C(9)–C(11) bond, the

* Structure and crystallographic numbering system, tables of atomic co-ordinates, isotropic thermal parameters, bond lengths, bond angles, anisotropic thermal parameters, and H-atom coordinates of (**2**); structure and crystallographic numbering system, tables of atomic co-ordinates, isotropic thermal parameters, bond lengths, bond angles, and H-atom co-ordinates of (**3**); 500 MHz proton NMR spectrum of (**2**) in $[^2H]_4$methanol, 500 MHz COSY-45 spectrum of (**2**), 500 MHz proton NMR spectrum of (**1**) in $[^2H]_4$methanol (15 pages) have been deposited at the Cambridge Crystallographic Data Centre. For details of the CCDC deposition scheme see 'Instructions for Authors (1990),' *J. Chem. Soc., Perkin Trans* 2, 1990, issue 1.

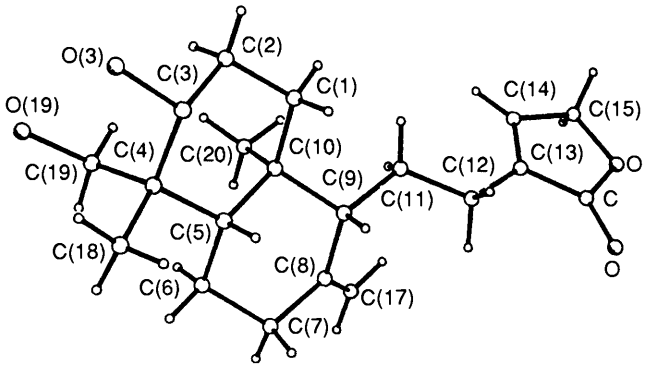

Figure 2. Projection from the crystal structure of 14-deoxyandrographolide (**3**).

coupling constants for H(9) are similar to those of neoandrographolide and indicate a similar conformation. In contrast, the conformation at the sp^2–sp^3 bond between C(11) and C(12) is more difficult to interpret. The subject of rotational isomerism about sp^2–sp^3 bonds has been a source of considerable theoretical interest and has been reviewed in detail.[8] The conformation at the sp^2–sp^3 bond is normally explained in terms of the double bond eclipsing or bisecting other groups, although it is not always clear whether the potential minima are sharp enough to discount torsional oscillation.[8] In the crystal structure of andrographolide, H(12) has dihedral angles of approximately 140° and 100° with H(11b) and H(11a) respectively [*i.e.* H(12) and C(9) are nearly eclipsed]. However, the observed coupling constants of 6.8 Hz between H(12) and H(11a)/H(11b) suggest a different conformational equilibrium at the C(11)–C(12) bond in solution. Based on our examination of molecular models, we propose that the observed coupling constants are best interpreted in terms of a mixture of conformations at the C(11)–C(12) bond in which the C(9)–C(11)–C(12)–C(13) dihedral angle lies within the range 120 and 240°. The observed coupling constants then represent an average of J_{gauche} and J_{trans} depending upon the relative energies of the rotamers or an average of all possible values in this range if torsional oscillation is occurring.

The remainder of the spectrum of (**1**) was assigned using the same procedure employed for neoandrographolide (**2**). Decoupling H(12) removed a long range coupling of 1.7 Hz to the doublet at δ 5.01 [H(14)]. A similar long-range coupling ($^4J_{trans} = -1.3$ Hz) is observed for propene.[7] Another decoupling experiment with irradiation of H(14) gave couplings of 6.1 Hz [H(15a) 4.45 ppm] and 2.0 Hz [H(15b) 4.16 ppm]. These were assigned as *cis* and *trans* protons, respectively, by comparison with model compounds.[7] The doublets at δ 4.12 and 3.37 were the diastereotopic methylene protons H(19a) and H(19b) ($J_{ab} = 11.0$ Hz), whilst H(17a) and H(17b) were broad singlets at 4.89 and 4.67 ppm. Assignments of the ring protons were again made with the aid of a COSY-45 experiment. Starting with $H(3)_{ax}$ (triplet at 3.41 ppm) a single cross peak at 1.80 ppm was identified in the COSY spectrum, consistent with $H(2)_{ax}$ and $H(2)_{eq}$ accidentally being almost magnetically equivalent. Addition of benzene gradually changed the multiplet structure of $H(3)_{ax}$ from a triplet to a quartet. The protons at 2.43 and 2.04 ppm must be $H(7)_{eq}$ and $H(7)_{ax}$ because of the multiplet structure and slope of the cross peak between them. Further cross peaks identify the vicinal neighbours $H(6)_{ax}$ (a quartet of doublets at δ 1.38) and $H(6)_{eq}$ (overlapped at 1.86 ppm). The benzene titration also allowed partial resolution of the overlapping multiplets at δ 1.35, revealing that $H(5)_{ax}$ is a doublet (δ 1.32) strongly coupled to $H(6)_{ax}$. Further assignments were made with the aid of NOE difference experiments. Irradiation of the high-field methyl [Me(20)] gave enhancements at 4.12 [H(19a) 2.60 ppm] [most likely H(11b)], 1.36 ppm [$H(6)_{ax}$], and 1.80 ppm [$H(2)_{ax}$ and probably $H(1)_{eq}$]. The lack of any enhancement at 1.30 ppm is consistent with $H(1)_{ax}$ being in this region and $H(1)_{eq}$ overlapping with $H(2)_{eq}$ and $H(2)_{ax}$ at 1.80 ppm. Previous assignments of *trans*-decalin and 10-methyl-*trans*-decalin[9] confirm that an axial proton is upfield of an equatorial proton in the absence of large substituent effects. Other NOE experiments indicated that the order of assignments for H(17a) and H(17b) was the same for both andrographolide and neoandrographolide. Irradiating H(17a) gave enhancements at H(17b) and $H(7)_{eq}$, whilst H(17b) gave enhancements at H(17a), H(12), and H(11b).

In addition to andrographolide and neoandrographolide, a third colourless crystalline solid was obtained from the extraction procedure. The 500 MHz proton spectrum suggested that this was a mixture of 14-deoxyandrographolide (**3**) and 14-deoxy-11,12-didehydroandrographolide (**4**). These compounds are known to have very similar polarities and have been separated by repeated chromatography on silver nitrate impregnated plates.[3d] However, this tedious procedure was not necessary for the present study; slow evaporation of an acetone solution of the mixture gave a single crystal of 14-deoxyandrographolide suitable for an X-ray diffraction study. A projection from the crystal structure is shown in Figure 2. This compound also crystallises as a monoclinic system with $P2_1$ space group. The cell constants are $a = 6.701(2)$, $b = 6.862(4)$ Å, $c = 19.282$ Å, $\beta = 92.41(3)°$, and $Z = 2$. As expected, the bond lengths and angles and the conformation of the lactone side-chain are similar to those of neoandrographolide. However, the lactone ring has a different orientation with respect to the C(9)–C(11)–C(12)–C(13) plane. There is also an intermolecular hydrogen bond between the hydroxy groups at C(19) and C(3). Full details of the structure determination are given in Table 1 and the Experimental section.

The structures of 14-deoxy-11,12-didehydroandrographolide (**4**) and 14-deoxyandrographolide (**3**) are so similar that an X-ray diffraction study of the second compound was not warranted. Furthermore, the NMR assignments of both molecules are very similar except for the C-11 vinyl group and adjacent protons. These assignments were therefore taken directly from the 500 MHz spectrum of the mixture using NOE measurements and a COSY-45 experiment. The NMR assignments for 14-deoxyandrographolide and 14-deoxy-11,12-didehydroandrographolide are included in Table 3 with those for andrographolide and neoandrographolide. It is worth noting the remarkably consistent chemical shifts for 14-deoxyandrographolide, 14-deoxy-11,12-didehydroandrographolide and andrographolide [*e.g.* Me(18), $H(3)_{ax}$, and H(19a) have chemical shifts within a range of 0.03 ppm in the three compounds].

The multiplet structure of the side-chain protons of 14-deoxyandrographolide is very similar to that found in neoandrographolide, implying a similar conformation for the side chain. For 14-deoxy-11,12-didehydroandrographolide a coupling constant of 10.1 Hz between H(9) and H(11) indicates a pronounced conformational preference at the C(9)–C(11) bond. An NOE difference experiment with irradiation of Me(20) produces an enhancement at H(11), implying that H(11) is in a *trans* relationship with H(9). This is similar to the conformation of andrographolide and neoandrographolide. For the C(11)–C(12) vinyl group a coupling constant of 15.8 Hz between H(11) and H(12) has been interpreted in terms of a *trans* configuration,[3d] whilst a NOE difference experiment with irradiation of H(14) gives an enhancement at H(12) and a much smaller enhancement at H(11), which suggests that the diene is in a *transoid* conformation.

Table 3. Proton chemical shifts (0.04 mol dm^{-3} in methanol at 298 K) of andrographolide (**1**), neoandrographolide (**2**), 14-deoxyandrographolide (**3**) and 14-deoxy-11,12-didehydroandrographolide (**4**) relative to methanol at 3.31 ppm. Assignments marked with a P are provisional.

Proton	(1)	(2)	(3)	(4)
$H(1)_{ax}$	1.30	1.08	1.26P	1.20P
$H(1)_{eq}$	1.80	1.80	1.80P	1.48P
$H(2)_{ax}$	1.80	1.63	1.74P	1.74P
$H(2)_{eq}$	1.80	1.46	1.74P	1.74P
$H(3)_{ax}$	3.41	0.96	3.38	3.40P
$H(3)_{eq}$	—	1.96	—	—
$H(5)_{ax}$	1.32	1.26	1.20P	1.20P
$H(6)_{ax}$	1.38	1.39	1.34	1.41
$H(6)_{eq}$	1.86	1.87	1.84	1.84
$H(7)_{ax}$	2.04	1.97	1.98	2.06
$H(7)_{eq}$	2.43	2.42	2.43	2.45
$H(9)_{ax}$	1.92	1.68	1.63P	2.38
H(11a)	2.63	1.79	1.75P	6.85
H(11b)	2.58	1.63	1.63P	—
H(12a)	6.85	2.40	2.39	6.16
H(12b)	—	2.10	2.09	—
H(14)	5.01	7.33	7.34	7.44
H(15a)	4.45	4.82	4.81	4.86
H(15b)	4.16	—	—	—
Me(16)	0.76	0.71	0.69	0.84
H(17a)	4.89	4.86	4.88	4.76
H(17b)	4.67	4.63	4.65	4.50
Me(18)	1.22	1.03	1.20	1.23
H(19a)	4.12	4.09	4.10	4.13
H(19b)	3.37	3.22	3.34	3.38
H(1′)	—	4.17	—	—
H(2′)	—	3.16	—	—
H(3′)	—	3.31	—	—
H(4′)	—	3.31	—	—
H(5′)	—	3.23	—	—
H(6′a)	—	3.85	—	—
H(6′b)	—	3.68	—	—

Experimental

Extraction Procedure.—*Andrographis paniculata* (334 g of dried whole plant) was homogenised and soaked in 95% ethanol (1 dm^3) for 24 h. This procedure was repeated three times and the combined ethanol extracts were reduced to a volume of 500 cm^3. After decolourisation with activated charcoal (28 g) the volume was reduced to 45 cm^3 and the solution was allowed to crystallise overnight at 4 °C, yielding crude andrographolide (1.5 g). This crude product was recrystallised from acetone. The filtrate was then evaporated to remove ethanol and extracted with chloroform (3 × 50 cm^3). The combined extracts were dried over anhydrous Na_2SO_4, reduced to a volume of 20 cm^3, and then chromatographed on neutral alumina (Brockmann Grade III) using chloroform and finally acetone as eluants. The first fraction was recrystallised from acetone, giving a mixture of 14-deoxyandrographolide (**3**) and 14-deoxy-11,12-didehydroandrographolide (**4**) (2.0 g). Fraction 2 contained neoandrographolide (**2**) (2.1 g) which was recrystallised from acetone.

X-Ray Structure Determinations.—Crystals of neoandrographolide (**2**) and 14-deoxyandrographolide (**3**) were obtained by slow evaporation of an acetone solution. Diffraction data were collected at 130K on a $P2_1$ diffractometer employing Mo-K_α radiation (λ = 0.710 69 Å) from a graphite monochromator. Details of the crystal parameters and the data collection and refinement are given in Table 1. Two possible space groups, $P2_1$ and $P2_1/m$ were indicated. Based on values for E^2-1, the non-centrosymmetric space group $P2_1$ was considered to be more likely. The structures were solved by direct methods using programs from SHELTXL (Revision 5.1) installed on a Data General Eclipse computer.[10] The crystal structure of neoandrographolide was refined using a blocked-diagonal least squares technique with 340 parameters and 2 018 reflections to yield an R value of 0.049. Non-hydrogen atoms were refined with anisotropic thermal parameters, whilst hydrogen atoms bonded to the carbon atoms were included at calculated positions using a riding model, with C–H of 0.96 Å and U_H = 1.2 U_C. The hydrogen atoms bonded to oxygen were located on a difference map and included in the refinement with an additional constraint on the O–H distance of 0.92(5) Å and isotropic thermal parameters fixed at U = 0.04 $Å^2$. The largest feature on a final difference map was 0.38 e $Å^{-3}$ in height. For 14-deoxyandrographolide, full-matrix least-squares refinement using 102 parameters and 893 reflections yielded an R value of 0.083. Due to the poorer crystal quality and paucity of data, isotropic thermal parameters were used for all the non-hydrogen atoms. Hydrogen atoms bonded to the carbon atoms were again included at calculated positions using a riding model, with C–H of 0.96 Å and U_H = 1.2 U_C. Hydrogen atoms bonded to oxygen were not included in the refinement. The largest feature on a final difference map was 0.44 e $Å^{-3}$ in height.

NMR Experiments.—Proton NMR spectra were obtained using a Nicolet NM-500 spectrometer, except for NOE measurements on the mixture of 14-deoxyandrographolide and 14-deoxy-11,12-didehydroandrographolide, which were obtained using a GE QE300 spectrometer. In each case the sample concentration was approximately 0.04 mol dm^{-3} in $[^2H]_4$methanol (99.99% ^2H). Measurements were taken at 298 K and the solvent methyl peak was used as a reference (3.31 ppm). Chemical shifts of overlapping peaks were taken from NOE difference or COSY-45 experiments. Typical conditions for acquiring proton spectra included a spectral window of 4 000 Hz, 16K data points and a 45° pulse. Modest resolution enhancement was applied using a double-exponential multiplication (DM = 4) and the data set was zero-filled to 32K (digital resolution 0.25 Hz per point). For the NOE experiments the resonance under examination was saturated for 4 s prior to a 90° read pulse. An exponential line broadening factor of 1.5 Hz was applied before zero-filling and transformation of the data. The COSY-45 spectra were obtained using a standard pulse sequence from the Nicolet software library. Typical conditions included a spectral window of 1 500 Hz, 1K data points for each of 128 $t1$ increments and a recycle time of approximately 2.3 s. Apodisation with a sine-bell multiplication and two orders of zero-filling in $t1$ gave a final matrix size of 512 × 512 words (digital resolution 3 Hz per point). After inspection the data set was symmetrized.

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