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A LIMONOID, PSEUDRELONE B, FROM *PSEUDOCEDRELA KOTSCHYII*

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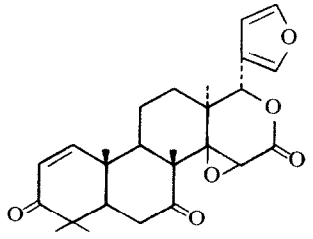
Key Word Index—*Pseudocedrela kotschyii*; Meliaceae; limonoids; pseudrelone B.

Pseudocedrela kotschyii is a small tree of a monotypic genus, widely distributed in the sub-Saharan savannah zones of Central Africa. It is very closely related to the better known genus *Entandrophragma*, the source of several valuable timbers, but differs from it in important botanical characters.

Ekong and Olagbemi [1, 2] have reported on the isolation of a variety of limonoids from the timber, including 7-deacetoxy-7-oxogedunin (**1**) and the pseudrelones A, B and C. Each of these consisted of a single limonoid alcohol esterified with a variety of acids, giving rise to a separable series of esters identified by subscript numbers. It has recently been shown that the pseudrelones A are 15-acyl derivatives of phragmalin (**2**), while pseudrelone C is a ring D-opened derivative of phragmalin (Nakanishi, K., personal communication) similar to procerin [3].



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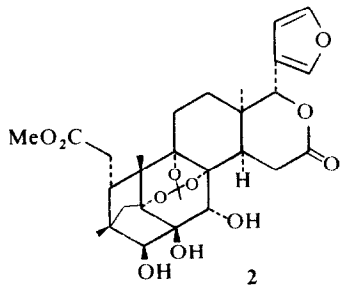

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We now report an extraction of the bark of *Pseudocedrela kotschyii* collected in the Upper Ogun Forest Reserve in Kwara State, Nigeria, in 1972. This gave only one limonoid which appeared to be identical (^{1}H NMR) with pseudrelone B_2 of Ekong and Olagbemi [2]. We therefore retain the name pseudrelone B for this substance. Pseudrelone B, mp 255–257°; $[\alpha]_D$ −70°, was found to have the molecular formula $C_{38}H_{46}O_{16}$ Examination of the spectra showed the presence of the usual furan ring (IR, ^{1}H NMR, ^{13}C NMR); two methyl ester groups; three other esters, identified as two acetates and an isobutyrate, and an orthoacetate (^{13}C NMR δ 117.6 singlet, ^{1}H NMR δ 1.78, three-proton singlet). These account for fourteen of the sixteen oxygen atoms, and with the usual C_{26} limonoid nucleus, for all the carbon atoms.

The fifteenth oxygen atom was present in a carbonyl group (^{13}C NMR δ 197.3, IR 1640 cm^{-1}), which appeared to be conjugated with the furan ring (UV λ_{max} 261 nm, ε 2.9 × 10^3).

Quantitative hydrolysis showed the presence of five equivalents of acid per mol; these being identified as the two methyl esters, and three equivalents of volatile acid, two of acetic acid and one of isobutyric acid. The orthoacetate did not hydrolyse, which suggested that it was not attached to the molecule through C-14 [3].

The evidence thus far suggests that pseudrelone B, like pseudrelone C, was a ring D-opened derivative of phragmalin, in which the 17-hydroxyl was oxidized to a ketone. The spectral features expected for the 3,2,30-triacyl grouping and for the 1,8,9-orthoacetate were found in the ^{1}H NMR and ^{13}C NMR spectra; the doublet carbon atoms were related to the coupled protons by off-resonance decoupling at two offset frequencies (C-3, δ 81.2; H-3, 5.11; C-30, 70.2; H-30, 6.58; C-2, 1, 8, 9, 88.5, 87.7, 84.0, 83.2; not necessarily respectively). The resonance for the ring A bridgehead carbon atom was found at the characteristically downfield position of δ 42.3, close to the position in all other known A-bridged limonoids [3]. The proton resonances due to this group were obscured by the two AB_2 systems due to the two ·CH·CH$_2$·COOMe groupings and were not detected.

The structure deduced so far was one oxygen atom short of the molecular formula of pseudrelone B and had two hydrogen atoms too many. The extra oxygen could not be present as a ketonic carbonyl group, as there was only one such group (^{13}C NMR, IR); it therefore seemed to be present as an ether linkage. This deduction was confirmed by the presence of two more carbon resonances in the C-O region of the ^{13}C NMR spectrum. One of these (δ 70.9) was present as a triplet in the off-resonance decoupled spectrum, the other (δ 72.0), as a doublet (coupled proton δ 3.63). The ether therefore formed a CH$_2$·O·CH· group which was not an epoxide, since this would give carbon resonances further upfield.

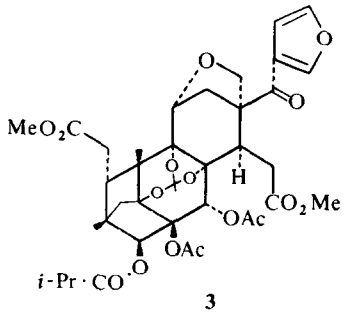
In accordance with this assignment, the proton spectrum, which was very clearly resolved in the methyl region, showed only two nuclear tertiary methyl groups, at δ 0.94, 1.58 (coupled carbons, δ 14.4, 25.8). The proton resonances relating to the oxide group were found near δ 4, although in deuterochloroform solution, they were partly obscured by the two carbomethoxy groups (δ 3.60, 3.64). One of the methylene protons was clearly visible as a doublet (δ 4.34, J = 7 Hz), one half of the other doublet could be seen (δ 3.74), while the other was concealed by the methoxy groups. This was confirmed by proton decoupling. The position of the methine

resonance was estimated from decoupling of the carbon spectrum to be δ 3.63, which would be concealed by the methoxy groups and this was supported by the proton integral which showed the presence of eight proton resonances in the δ 3.6 region, two methoxy groups, one methylene proton, and the missing methine proton. Running the spectrum in a judicious mixture of deuteropyridine and deuterochloroform shifted the resonance positions sufficiently to reveal both the partly concealed methylene proton and the methine proton, which appeared as the X part of an ABX multiplet, splitting 16.6 Hz.

It remained to discover which carbon atoms were joined in the ether linkage. Examination of a model showed that the methine end of the ether bridge could only be at C-11, assuming a phragmalin skeleton. The only other methylene group in the molecule which was adjacent to a second methylene group and thus would give rise to an ABX multiplet on oxygen substitution, was that at C-12. This could not be linked in a sterically credible oxide ring with any of the three methyl groups, one of which must form the methylene end of the ether ring. A linkage from C-11α to C-13A, or from C-11α or β to C-10A was sterically possible.

One of the α-furan proton resonances in the spectrum of pseudrelone B was unusually far downfield at δ 8.05 (normally not beyond 7.4); the coupled carbon atom was also unusually far downfield (δ 147.2, usually 140). This may be partly accounted for by the presence of a C-17 carbonyl group; however this was not the complete answer. Alkaline hydrolysis of pseudrelone B gave a dicarboxylic acid with a sharp proton spectrum, in which the positions of the three furan resonances remained unchanged. Reduction of the ketonic group in this acid with borohydride, followed by re-esterification with diazomethane and acetic anhydride, removed the δ 8.0 resonance and replaced it with two resonances of equal intensity, one at 8.1 and the other at 7.9; the other furan resonances were not altered. These two bands, due to the production in approximately equal amounts of the two stereoisomers at C-17, were still much further downfield than any furan resonances we have seen before. This observation would be adequately explained if the downfield shift was mainly due to the presence of an oxygen at C-13A, the substitution at C-17 making only a small additional effect. The presence of the ether oxygen in the alternative position at C-10A would leave the shift in resonance frequency of the furan proton unexplained. We therefore propose for pseudrelone B the structure **3**, containing a phragmalin nucleus with an opened ring D and the 17-hydroxyl oxidized to a ketone, and with an oxide ring extended from C-11α to C-13A. The splitting of the 11β proton resonance in pseudrelone, referred to above, is 16.6 Hz. In utilin (**4**), which has a similar structure also containing an 11α-oxygen function [4], the splitting of the 11β proton resonance is 16 Hz. This agreement, although gratifying, may be largely coincidental, as the different location of the orthoacetate function in the two compounds, and the presence of the oxide ring in pseudrelone, have unpredictable effects on the stereochemistry of ring C.

The same conclusion for the location of the oxygen ring can also be arrived at using a different argument. In all limonoids, except those oxidized at C-4, the protons on the methyl groups joined to C-4 resonate markedly upfield of those on the remaining nuclear methyl groups.



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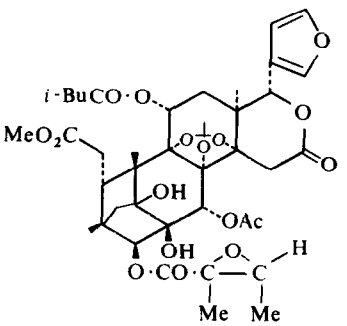

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In phragmalin triacetate, the nuclear methyl groups resonate at δ 0.96, 1.03 and 1.21, in pseudrelone C at 0.93, 1.15 and 1.20, and in pseudrelone B at 0.94 and 1.58. The highest field resonance, which is nearly constant throughout, we assign to the 4-methyl group, which is therefore seen to remain in pseudrelone B. Only one of these resonances shifts notably between pseudrelone C and phragmalin acetate; as the only significant difference is the opening of ring D we assign the shifting resonance to the C-13 methyl group, and the one constant at δ 1.20 to the C-10 methyl group. In pseudrelone B only one methyl group resonated at the same frequency as in phragmalin, one resonance had shifted notably and the third had disappeared, consequent on oxidation of the methyl group.

In the product of borohydride reduction of pseudrelone, described above, the nuclear methyl groups resonated at δ 0.93 and 1.62; the presence of two isomers at C-17 produces no doubling of the bands. Therefore, as expected, reduction of the 17-carbonyl group produced no detectable effect on the C-4 methyl group, and only a slight shift from δ 1.58 to 1.62, in the resonance of the second methyl group. This was consistent with location of the methyl group on C-10, but not with location on C-13, where it would surely suffer a much larger shift. On the other hand, in the reduced compound one of the resonances of the methylene group protons was considerably shifted, from δ 3.68 in pseudrelone B to 4.0 in the reduction product, while the other was unchanged at 4.34. This was consistent with the presence of the methylene group at C-13A; but not with its presence at C-10A.

Considering now the location of the methine end of the oxygen bridge, the ^{13}C NMR spectrum of phragmalin showed five methylene carbon resonances, those of the ring A bridgehead, C-6, C-13, C-11 and C-13 at δ 40.2, 33.3, 29.15, 26.6 and 25.4. We assign these as shown, by comparison with other derivatives; the C-12 and C-11 resonances are always upfield of any others unless C-11

or C-12 is substituted. As anticipated, the spectrum of pseudrelone B showed only four methylene resonances at δ 42.3, 33.9, 33.9 and 31.6. We assign the first of these to the ring A bridge methylene; the second and third to the two similarly substituted $CH_2 \cdot CO_2Me$ methylene carbons. The resonances due to C-11 and C-12 were shifted or lost, showing substitution at one of these positions.

Substitution of an oxygen at C-11 would be expected to produce a considerable shift of the C-10 methyl resonance position; substitution at C-12 should make a much smaller effect. It is not possible to predict the size of the effect due to lack of suitable models and uncertainty about the stereochemistry of ring C, but the observed shift of the resonance position from δ 1.2 in phragmalin to 1.58 in pseudrelone B was much too large to be probable as a result of oxygen substitution at C-12, while it was within the range of possibility for introduction of an oxygen atom at C-11 [5].

Finally, we consider the probable location of the acyl groups. Pseudrelone B contained two acetates and an isobutyryl group, which are attached at C-3, C-2 and C-30. One of the acetate methyl groups resonated at δ 2.16, the other at 2.02. In phragmalin triacetate, the acetate methyls resonated at δ 2.23, 2.13, 1.93; by comparison with partially acetylated derivatives these can be assigned to the acetates at C-3, C-2 and C-30, respectively. It therefore seems probable that pseudrelone B has the isobutyrate at C-3 and acetates at C-2 and C-30. This would be in line with the general pattern of substitution in poly-oxygenated fissinolide derivatives; when only one more complex acyl group is present in a molecule this is usually at C-3, with acetates at the other positions.

EXPERIMENTAL

Isolation of pseudrelone B. The milled bark of *Pseudocedrela kotschyii* (10 kg, collected by the author in the Upper Ogun Forest Reserve, Kwara State, Nigeria) was percolated with refluxing hexane. The insoluble portion of the extract was washed with Et_2O and crystallized from MeOH to give pseudrelone B (150 mg) (mp 255–7°, $[\alpha]_D^{20}$ −70°, identical (^{1}H NMR, IR) with the pseudrelone B_2 of Ekong and Olagbemi [2]. (Found: C, 60.1; H, 6.5; M^+ 758. Calc. for $C_{38}H_{46}O_{16}$: C, 60.1; H, 6.1%). ^{13}C NMR: δ 197.3*s*, 176.8*s*, 173.1*s*, 172.0*s*, 169.8*s*, 167.9*s*, 147.2*d*, 143.0*d*, 124.1*s*, 117.6*s*, 110.5*d*, 88.5*s*, 87.7*s*, 84.0*s*, 83.2*s*, 81.2*d*, 72.0*d*, 70.9*t*, 70.2*d*, 56.2*s*, 51.7, 2*q*; 51.2*s*, 48.5*d*, 45.8*s*, 42.3*t*, 35.8*d*, 33.9, 2*t*; 33.7*d*, 31.6*t*, 25.8*q*, 21.7*q*, 21.2*q*, 20.9*q*, 19.8*q*, 18.4*q*, 14.4*q*. ^{1}H NMR: δ 8.05*m*, 7.36*m*, 6.8*m*, 6.58*s*, 5.11*s*, 4.34*d* (J = 7 Hz), 3.68*d* (J = 7 Hz); 2.16, 3H*s*; 2.02, 3H*s*; 1.78, 3H*s*; 1.58, 3H*s*; 1.27, 1.16, 2*d* (J = 6.5 Hz, isopropyl); 0.94, 3H*s*. ν_{max}^{Nujol} cm^{-1}: 1740, 1720, 1640, 1490, 900, 870, 745. UV λ_{max} nm: 261 (ε 2.9 × 10^3).

Quantitative hydrolysis. Pseudrelone B (26.5 mg) was refluxed with 0.1 M NaOH soln (5 ml) in MeOH (10 ml). Titration in the usual way showed the presence of 4.23 mol of total acid and 2.05 mol of volatile acid, identified spectroscopically [7] as acetic and isobutyric acids in the ratio 2:1.

Qualitative hydrolysis and reduction. Pseudrelone B (50 mg) was hydrolysed with aq. methanolic NaOH. The ^{1}H NMR spectrum of the product, which was not purified, lacked the resonance due to the several ester groups, but was otherwise similar to that of pseudrelone B. This was dissolved in dil NaOH soln and treated with $NaBH_4$ (200 mg). The product was esterified with CH_2N_2 and then with Ac_2O–Py to give a mixture of dihydro derivatives, which was not purified. The ^{1}H NMR spectrum was similar to that of pseudrelone, with some doubling of bands; δ 8.15, 7.95 (doubled band); 7.4*m* (widened band); 6.8*m*, 6.58*s*, 5.1*s* (not sharp), 4.34*d* (J = 7 Hz), 4.0*d* (J = 7 Hz), 3.72, 3.64, 2.17, 1.78, 1.62, 0.93; IR cm^{-1}: 1640 band lacking; UV λ_{max} nm: 209 (ε 6.2 × 10^3).

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