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The Constituents of *Carapa guianensis* Aubl. and their Biogenetic Relationship

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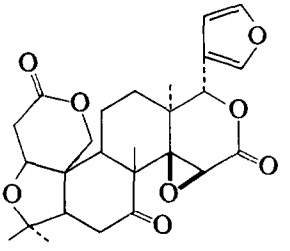
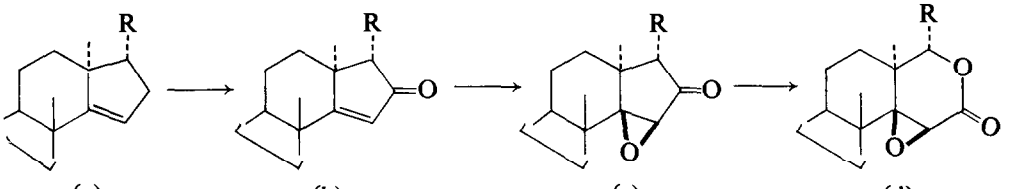
Seven compounds, namely, epoxyazadiradione (III), 6α-acetoxy-epoxyazadiradione (VI), 6α-acetoxygedunin (Va), 6α-hydroxygedunin (Vb), 7-deacetoxy-7-oxogedunin (IVb), andirobin (VII), and methyl angolensate (VIII), were isolated from *Carapa guianensis* Aubl., three of which, VI, Va, and Vb are new compounds. The biogenetic implication of the isolation of such a series of compounds from one and the same plant is discussed in view of oxidative degradation and transformation sequences.

The limonoids are a group of mostly bitter C_{26} degraded triterpenes, exemplified by limonin. This compound was the first in this series to be extensively studied. One of its characteristic features is the presence of a 6-membered ring D epoxylactone. Its formation has been rationalized by an attractive biogenetic sequence involving a Δ^{14}-5-membered ring D precursor (a). Such a compound would then undergo allylic oxidation to Δ^{14}-16-ketone (b) and epoxidation (c), followed by a Baeyer–Villiger-type oxidation producing the required 6-membered ring epoxylactone (d).



Limonin



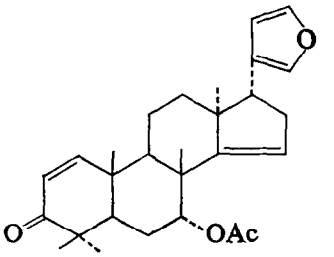
(a) (b) (c) (d)

In previous publications we have reported the isolation of four limonoids from *Melia azadirachta* L. (*2*), namely, azadirone (I), azadiradione (II), epoxyazadiradione (III) as well as the known compound gedunin (IVa), which all could be looked upon as limonoids following the increasing oxidation level represented in the above biogenetic sequence (*4*) [compare (a) → (b) → (c) → (d)]. We have also reported chemical transformations of one compound to another (*2*), as well as the conversion of epoxyazadiradione (III) to gedunin (IVa), by treatment with perbenzoic acid. These reactions further support the proposed sequence (*4*).

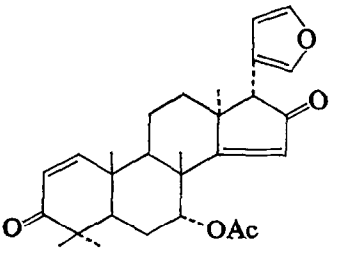
Furthermore, in the same group of compounds the cleaved ring B leading to the types of compounds best exemplified by andirobin (*1*) VII and methyl angolensate (*6*) VIII can now be conceived as arising, similarly, from a Baeyer–Villiger-type oxidation leading to a ring B ϵ-lactone. Opening of this lactone to the corresponding 8-hydroxy-7-carboxylic acid, followed by dehydration toward C-30 would produce the double bond exocyclic to ring C (*7*). The prerequisite for such a sequence would be a C-7 carbonyl-bearing compound. Indeed such a compound is known to occur in nature, and is exemplified by 7-deacetoxy-7-oxogedunin (*8*) IVb. This compound was actually converted in the laboratory to 14,15-deoxyandirobin (*9*). The latter, in turn, readily gives methyl angolensate through a prior hydroxylation at C-1, followed by Michael addition from the β side to the C-14 double bond of ring D producing the expected bicyclo-(3,3,1)-oxanonane (*10*).

We now report the isolation of a series of compounds which represent the different stages of the hypothetical pathway leading to methyl angolensate (VIII) from a single plant source, *Carapa guianensis* Aubl. (*1*). These compounds are epoxyazadiradione (*2*) III, 6α-acetoxyepoxyazadiradione VI, 6α-acetoxygedunin Va, 6α-hydroxygedunin Vb, 7-deacetoxy-7-oxogedunin (*8*) IVb, andirobin (*1*) VII and methyl angolensate (*6*) VIII.

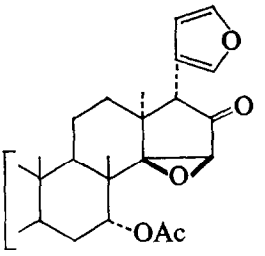
Whereas compounds III, IVb, VII, and VIII have been described earlier and could be, therefore, identified by comparison with authentic samples, the structure of 6α-acetoxyepoxyazadiradione VI had to be determined. Analysis of its NMR spectrum showed doublets at δ 7.10 and 5.93 ($J = 10$ Hz) related to the 1-H and 2-H, respectively, while the assignment of the other protons was as follows: the epoxidic 15-H singlet at δ 3.38, 17-H singlet at δ 3.85, and furanic α, α', and β protons, δ 7.54, 7.38, and 6.22, respectively. The location of the signals given above are in agreement with those assigned for the same protons in epoxyazadiradione (*2*) III. However, in the NMR spectrum of the latter the 7β-H geminal to the acetoxy group is a triplet at δ 4.68, whereas in VI, the signal corresponding to the same proton is a doublet at δ 5.01 ($J = 3$ Hz), indicating the presence of a substituent which had to be at C-6. The nature of this substituent was determined through its NMR spectrum, in which two CH_3 signals corresponding to two acetoxy groups at δ 1.96 and 2.01 were observed, the former being related to the C-7α OAc while the latter to the C-6 OAc group; the 6-H proton being the doublet of doublets at δ 5.36 ($J = 13$ and 3 Hz). Having placed the second acetoxy group in the molecule, it remained to determine its orientation. From earlier work on the meliacins, the C-7 OAc group was shown to be α-axial (*11*). Since the coupling constant between the 7β-H and the 6-H is small and of the order of 3 Hz, a small dihedral angle exists between these two protons, a situation which could arise only from a 7β-equatorial and a 6β-axial proton. Furthermore, since the 5α-H is axial and thus in a trans diaxial orientation with respect to the 6β-H, maximal coupling



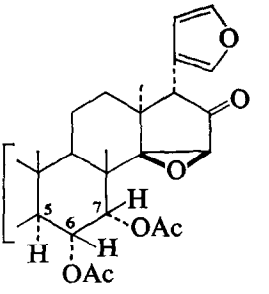
I



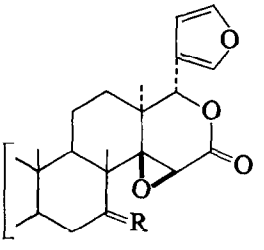
II



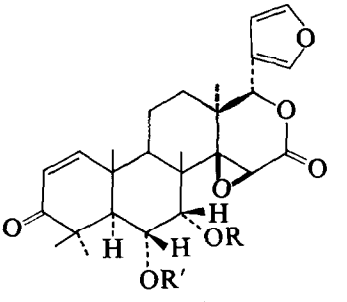
III



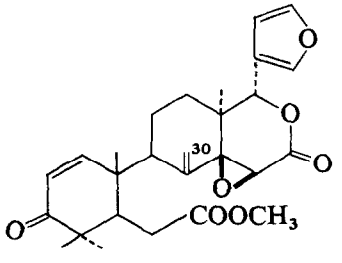
VI



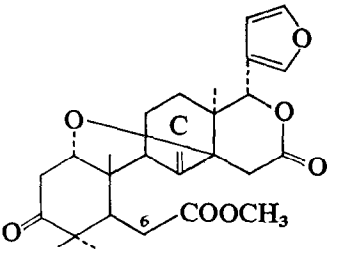
IV a. R = α-OAc, β-H
b. R = O



V a. R = R′ = Ac
b. R = Ac, R′ = H
c. R = R′ = H



VII



VIII

would be expected between these two protons, and indeed the observed coupling constant is 13 Hz; as to the 5α-H it is a doublet at δ 2.47 ($J = 13$ Hz). In order to confirm that the signal pattern of the 6β-H is due to coupling with the 7α-H and 5α-H, irradiation at the frequency corresponding to the center of the 7α-H signal was performed, leading to the collapse of the 6β-H doublet ($J = 13$ Hz). A similar collapse was also observed upon irradiation at the frequency of the signal of 5α-H, resulting in a doublet ($J = 3$ Hz).

The NMR spectrum of compound Va shows the following signals: 6α-H doublet of doublets at δ 5.32 ($J = 13$ and 3 Hz), 7α-H doublet at δ 4.93 ($J = 3$ Hz), 5α-H doublet at δ 2.54 ($J = 13$ Hz), 1H doublet at δ 7.13 ($J = 10$ Hz), 2-H doublet at δ 5.98 ($J = 10$ Hz), 15α-H singlet at δ 3.64, αα′, and β furanic protons multiplets at δ 7.46 and 6.39, respectively. The 6α and 7α-Me signals of the two acetoxy groups were observed as singlets at δ 2.05 and 2.17, respectively. All these signals are in good agreement with those of the same protons observed for 6α-acetoxyepoxyazadiradione VI, the only difference being in the location of the 17α-H signal. In compound Va it is shifted to lower field due to the proximity of the ether oxygen of the ring D lactone and is a singlet at δ 5.66.

Supporting chemical evidence for the structure of compound VI was obtained through its quantitative conversion to 6α-acetoxygedunin (Va) by treatment with perbenzoic acid (Baeyer–Villiger oxidation) (*2b*).

Another compound to be isolated was 6α-hydroxygedunin Vb, and its structure was inferred from its NMR spectrum: 6β-H doublet of doublets at δ 4.30 ($J = 13$ and 3 Hz), 7β-H doublet at δ 4.78 ($J = 3$ Hz), as well as by its conversion upon acetylation to IVb.

The biogenetic relationship between IVb, VII, and VIII is evident, and this is the first instance to be reported where the three compounds were isolated from one and the same plant (the seeds), thus, very strongly supporting the postulated biogenetic pathway described above, leading ultimately to the most oxidatively degraded product—methyl angolensate VIII. The isolation of III, VI, Vb, and Va may be looked upon as intermediates in the biogenetic pathway leading to compounds such as 6-hydroxy or 6-acetoxy methyl angolensate (*12*).

EXPERIMENTAL

Melting points were taken on a Fisher–Johns apparatus. Optical rotations refer to $CHCl_3$ solutions. Infrared spectra were recorded on a Perkin–Elmer Infracord model 137 spectrophotometer equipped with a NaCl prism, and were determined in KBr pellets. Ultraviolet spectra were recorded on a Cary 14 in EtOH solutions. Nuclear magnetic resonance spectra were recorded on a Varian A-60 and Brucker HFX-10 90 MHz spectrometers, for 5–10% solutions in $CDCl_3$, in the former case TMS was used as internal standard. Molecular weights and mass spectra were determined using an Atlas CH4 instrument by Mr. S. Gattegno. Elemental analyses were performed in our analytical laboratory under the direction of Mr. R. Heller.

Isolation procedure. The peeled seeds of *Carapa guianensis* Aubl. (8200 g) were cut into small pieces and defatted by extraction with cold petroleum ether (bp 40–60°). The defatted material was then extracted with $CHCl_3$ to give a crude product (353 g) from which andirobin VII and 7-deacetoxy-7-oxogedunin IVb were isolated by column chromatography on silica-H and elution with benzene–$CHCl_3$. Further extraction of

the seeds with MeOH afforded a viscous mass which after washing with water and drying gave a crude product (64 g). Column chromatography of this material on silica-H and elution with benzene yielded the compounds as follows:

Epoxyazadiradione III. The fractions containing epoxyazadiradione III as their major constituent were combined and further purified by preparative thick-layer chromatoplates irrigated with petroleum ether–EtOAc (7:3). Pure III (15 mg) was crystallized from MeOH and found to be identical with an authentic sample (*2*).

6α-Acetoxyepoxyazadiradione VI. The combined fractions containing VI as their major constituent yielded pure crystalline material from preparative thick-layer chromatoplates irrigated with petroleum ether–EtOAc (6:4); mp 167–169°; $[\alpha]_D$ +40° (c 0.1); λ_{max} 225 nm (ϵ 11,000); ν_{max} 1751 ($\alpha\beta$-epoxycyclopentenone), 1736 (acetates), 1680 (cyclohexenone), and 886 (furan) cm^{-1}. (Found: M^+ 524. $C_{30}H_{36}O_8$ requires: MW 524.59.)

Andirobin VII. The fractions showing the presence of andirobin VII as one spot on chromatoplates (petroleum ether–EtOAc 1:1) afforded a pure crystalline compound from MeOH. It was found to be identical with an authentic sample of VII.

6α-Acetoxygedunin Va. The other set of fractions showing Va as one spot on chromatoplates irrigated with petroleum ether–EtOAc (1:1) were combined and the product crystallized from acetone–MeOH, mp 270–273°; $[\alpha]_D$ +141° (c 1.0); λ_{max} 227 nm (ϵ 10,000); ν_{max} 1740 (acetates and $\alpha\beta$-epoxy-δ-lactone), 1680 and 886 cm^{-1}. (Found: C, 66.36, H, 6.65; M^+ 540; $C_{30}H_{36}O_9$ requires: C, 66.65; H, 6.71%; MW 540.59.)

7-Desacetoxy-7-oxogedunin IVb. The fractions containing IVb as a single spot on chromatoplates irrigated with petroleum ether–EtOAc (1:1) were combined. Pure IVb crystallized from MeOH and was found to be identical with an authentic sample.

6α-Hydroxygedunin Vb. The fractions containing Vb as major constituent on chromatoplates irrigated with petroleum ether–EtOAc (1:1) were combined and pure Vb was obtained by crystallization from a hexane–$CHCl_3$ mixture, mp 175–177°; $[\alpha]_D$ +100° (c 0.1); λ_{max} 225 nm (ϵ 11,000); ν_{max} 1740, 1680, and 886 cm^{-1}. (Found: M^+ 498; $C_{28}H_{34}O_8$ requires: 498.55.)

Methyl angolensate VIII. The fractions showing on chromatoplates irrigated with petroleum ether–EtOAc (2:3) VIII as major component were combined. Further purification by preparative thick-layer chromatoplates irrigated with the same solvent mixture yielded pure VIII; crystallized from a hexane–$CHCl_3$ mixture and was found to be identical with an authentic sample.

Conversion of 6α-acetoxyepoxyazadiradione VI to 6α-acetoxygedunin Va. Compound VI (10 mg) in dry benzene (5 ml) was treated with a solution of perbenzoic acid in the same solvent (1 ml; 60 mg per ml) for 4 hr at room temperature. After the usual work-up the $CHCl_3$ layer was evaporated to dryness (*2b*). The crude product showing a single spot on chromatoplate irrigated with petroleum ether–EtOAc (1:1) crystallized from MeOH, mp 271–273 to yield Va; found identical in all respect with an authentic sample. This reaction is practically quantitative.

6α,7α-Dihydroxy-7-desacetoxygedunin Vc. To 6α-acetoxygedunin (0.15 g) MeOH-KOH 10% (10 ml) was added and the mixture heated to reflux for 10 min. After the usual work-up and neutralization with AcOH, the precipitate was washed with water, dried, and chromatographed on a Silica-H column, and eluted with $CHCl_3$-EtO_2 (4:1) to yield pure Vc (82 mg) crystallized as needles from acetone–hexane, mp 285–287°;

λ_{max} 225 nm (ϵ 10,800); ν_{max} 3470 (OH), 1740, 1680, and 886 cm^{-1}. (Found: C, 68.41; H, 6.85; M^+ 456; $C_{26}H_{32}O_7$ requires: C, 68.40; H, 7.07%; MW 456.52.)

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