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LIMONOIDS OF BIOGENETIC INTEREST FROM *MELIA AZADIRACHTA* L.[1]

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Abstract—The structure elucidation of three new limonoids of the meliacin type, azadirone (I), azadiradione (II) and epoxyazadiradione (III), as well as the isolation from the same plant *Melia azadirachta* L. of gedunin (IVa), having a ring D lactone, are described. Their structural relationship is analysed in view of a possible chemical degradation through stepwise oxidation and transformation in nature. Compounds having lactones in ring D and A, the latter being seven membered as in obacunone and nomilin, have been prepared to demonstrate further applications of this type of oxidative degradations.

ONE of the outstanding processes of chemical degradation in nature is through stepwise oxidation and transformation. Thus in order to account for an epoxylactone in ring D of limonin, an attractive biogenetic sequence of oxidative transformations had been postulated several years ago.[2] It was suggested then, that this epoxylactone could have been formed from a compound having a 14–15 double bond which upon allylic oxidation would give a Δ^{14}-16-ketone whereby subsequent epoxidation would lead to the corresponding 14β-15β-epoxy-16-keto derivative. This compound through a Baeyer-Villiger oxidation would then lead to the desired epoxylactone. We would like to present now the detailed account of the isolation from *Melia azadirachta* L. of the three compounds azadirone (I), azadiradione (II) and epoxyazadiradione (III), as well as that of the known gedunin (IVa),[1] which all can be looked upon as the various steps of the suggested biogenetic pathways leading in nature to a ring D epoxylactone. All these compounds have been interconverted by stepwise oxidation, the last taking place through a Baeyer-Villiger oxidation of epoxyazadiradione (III) to gedunin (IVa). These compounds are therefore tetranor-triterpenoids of the meliacin type (limonoids).

Further application of this type of oxidative degradation to ring A, in this series, producing a seven membered ring A lactone as is the case in the naturally occurring obacunone[3] and nomilin[4] type compounds, was observed when 1,2-dihydroepoxyazadiradione (VIIa) was converted to 1,2-dihydro-7α-obacunyl acetate (XI).[5] Such a conversion may well open the way and lead to additional degradations of the molecule as observed in compounds of the limonoid group.

The three compounds azadirone (I), azadiradione (II) and epoxyazadiradione (III), show absorption maxima in the UV at 225 nm, which are absent in the corresponding 1,2-dihydro-derivatives V, VI and VIIa obtained by the respective hydrogenation of I, II and III over $Pd/CaCO_3$. The loss of this chromophore upon hydrogenation, replaced however, by an absorption at 217 nm due to the furan, is associated with a shift of the CO band in the IR from 1680 to 1710 cm^{-1}. Identification of this chromophore as a cyclohexenone (in the three compounds) is in accord with the proton resonance signals of the 1-H and 2-H. These protons display two sharp doublets of

TABLE 1. CHEMICAL SHIFTS OF PROTONS OF THE COMPOUNDS (δ UNITS)

Compound	1-H	2-H	7β-H	15-H	17β-H	Furan α-H	Furan β-H	Ac	Me groups
I	7·18 d	5·83 d	5·35 t	5·24 t		7·35 m	6·30 m	1·97	0·81, 1·10(2), 1·20, 1·23
	J 10 Hz					7·25 m			
II	7·17 d	5·83 d	5·32 t	5·83 s	3·43 s	7·45 m	6·28 m	1·95	1·03, 1·08(s), 1·19, 1·35
	J 10 Hz								
III	7·10 d	5·76 d	4·68 t	3·33 s	3·83 s	7·52 m	6·18 m	1·98	1·00, 1·02(2), 1·19, 1·20
	J 10 Hz					7·35 m			
V			5·30 t	5·85 s	3·40 s	7·42 m	6·26 m	1·95	1·02(2), 1·03, 1·10, 1·30
VI			5·35 t	5·25 t		7·32 m	6·15 m	1·95	0·85, 1·00(3), 1·20,
						7·20 m			
VIIa			4·70 t	3·38 s	3·86 s	7·52 m	6·18 m	2·02	1·02(3), 1·06, 1·18
						7·35 m			
VIIb			3·05 t	3·55 s	3·87 s	7·50 m	6·20 m		1·0, 1·02, 1·05(3)
						7·38 m			
VIIc				3·58 s	3·76 s	7·50 m	6·20 m		0·86, 1·08, 1·20, 1·22(2)
						7·38 m			

Compound	at 4α	4β	8	10	13
VIIIa	1·03	0·97	1·07	1·11	1·02
VIIIb	1·03	1·00	1·11	1·11	1·11

Compound	1-H	2-H	7β-H	15-H	17β-H	Furan α-H	Furan β-H	Ac	Me groups
VIIIa	3·45 d	3·66 d	4·70 t	3·43 s	3·91 s	7·58 m	6·25 m	2·07	
	J 4·5 Hz					7·41 m			
VIIIb	3·45 d	3·66 d	3·62 t	3·61 s	3·91 s	7·66 m	6·26 m		
	J 4·5 Hz					7·41 m			
XI			4·53 t	3·53 s	5·65 s	7·45 m	6·39 m	2·17	1·43, 1·41, 0·97, 1·00, 1·23
	3α-H	7β-H	15-H	16-H	17-H				
XII	3·19 m	4·87 t	3·45 s	4·37 d	2·64 d	7·51 m	6·36 m	2·11	0·77, 0·89, 1·01, 0·91, 1·25
				J 4·5 Hz		7·40 m			
XIII	3·31 m	5·29 t	5·41 d	4·96 dd	2·62 d	7·45 m	6·39 m	1·91	0·78, 0·91, 0·90, 0·90, 1·21
			J 1·0 Hz	*J* 8·5 and 1·0 Hz	*J* 8·5 Hz				
XIV		4·83 t	3·46 s	4·36 d	2·66 d	7·49 m	6·31 m	2·13	1·07, 1·07, 1·01, 1·01, 1·28
				J 4·5 Hz		7·37 m			
XV	4·59 m	4·84 t	3·45 s	4·38 d	2·67 d	7·45 m	7·37 m	2 × 2·05	0·78, 0·85, 1·03, 0·95, 1·27
				J 4·5 Hz		7·40 m		2·12	
XVI	4·60 m	5·21 t	5·33 d	6·10 dd	2·90 d	7·43 m	6·37 m	2 × 2·00	0·78, 0·88, 0·97, 0·90, 1·21
			J 1·0 Hz	*J* 8·5 and 1·0 Hz	*J* 8·5 Hz			2·07	

the AB type at about δ 7·1 and 5·8 respectively (J = 10 Hz). Absence of additional splitting suggests that the system, which is an αβ-unsaturated ketone, could be placed in ring A, provided that the γ-C atom is tertiary. These doublets are missing in the NMR spectra of the corresponding dihydroderivatives. I, II and III also show a band in their respective IR spectra at about 1738 cm^{-1} associated with the acetate group at C-7. The protons of this group resonate between δ 1·95–1·98 (singlet, for 3 × H) while the 7β-H appears in each compound as a triplet (1/2 W ~ 7 Hz), the location depending on the nature of the substituent at C-15 (Table 1). The presence of this functional group is also manifested in the mass spectrum of each, by the respective fragments *m/e* 376 for I, 390 for II and 406 for III, in accord with M^+-60. Prolonged hydrogenation of III in aqueous dioxane with $Pd/CaCO_3$ in the presence of NaOH yielded the corresponding 1,2-dihydro-7α-hydroxy derivative VIIb in the NMR spectrum of which the 7β-H appears at δ 3·05 (triplet 1/2 W ~ 6 Hz); upon oxidation of VIIb to the 7-keto derivative VIIc this signal disappears, and the new CO function is manifested by a band in the IR at 1710 cm^{-1} consistent with its location in a saturated 6-membered ring. This CO function replaces the acetate group of the original compound. The axial α-orientation of the 7-Ac group is derived from the signal of the geminal 7β-H being a triplet indicating its equatorial conformation. As was shown to be the case in the meliacins,[6] the position of the 7β-H is influenced by the C-15 substituent, and *vice-versa*. It is now observed that whereas in VIIa the 15-H resonates at δ 3·38, in the corresponding 7α-hydroxy derivative VIIb this signal appears at δ 3·55, whereas following oxidation to the 7-keto (VIIc) the signal is at 3·58. Similarly the chemical shift of the 7β-H shifts from δ 4·68 in III (14β, 15β-epoxy group) to 5·32 in compound II (14,15-double bond). A similar interdependence has been also observed for gedunin (IVa),[6] cedrelone[7] and grandifolione,[8] as well as for other compounds of this series. From the similarity of effect with that of grandifolione, it may be concluded that the relative orientation of 7-H and 15-H in III is *anti*, and since the former is β-oriented the latter is α and the 14,15-epoxide is therefore β-oriented.

In all the compounds having a 16 ketone (II, III, V, VII) the resonance signals assigned to the 15-H and 17-H are singlets, the former being distinguished by its sharpness.

The interrelationship between compounds I, II and III has been performed by the oxidation of azadirone (I) with selenium dioxide in aqueous dioxan to azadiradione (II), which upon hydrogenation gave the crystalline 1,2-dihydroazadiradione (V) used for comparison purposes. Furthermore, 1,2-dihydroepoxyazadiradione (VIIa) was converted upon reduction with chromous chloride to the same 1,2-dihydroazadiradione (V).

The electron impact fragmentation pattern of this series of compounds is consistent with the assigned structures. In addition to the molecular ion peak, the simple fragmentation arising from the furan ring is observed in all cases leading to the loss of the fragment *m/e* 81. Also cleavage of the C-15 C-16 bond in the 16-ketone gives rise to loss of the fragment *m/e* 108 which is observed in the mass spectra of all the 16-ketone bearing compounds.

The absolute configuration assigned to I, II and III is in accordance with circular dichroism measurements. If the chromophore of an αβ-unsaturated ketone resides in a chiral environment, a circular dichroic absorption is observed for the $n \rightarrow \pi^*$

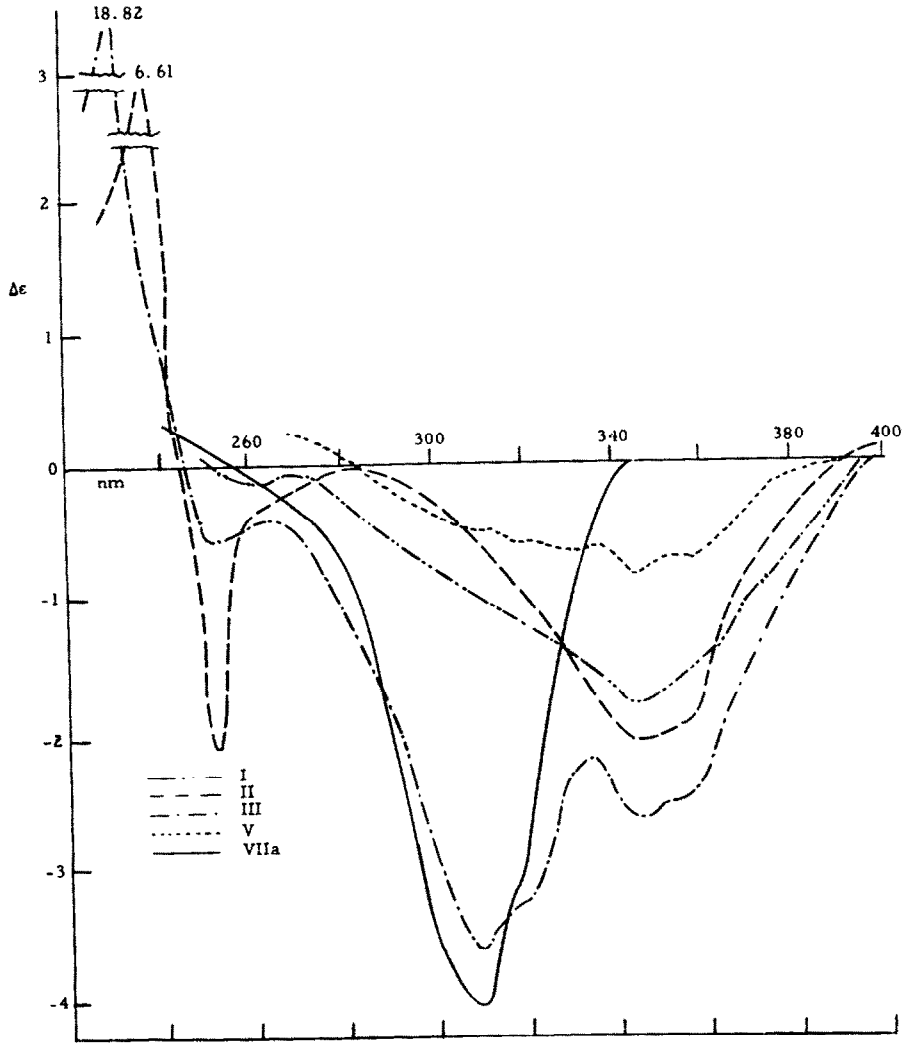
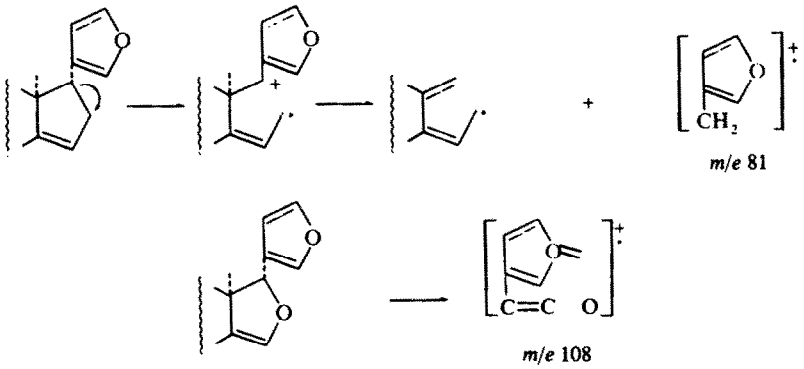
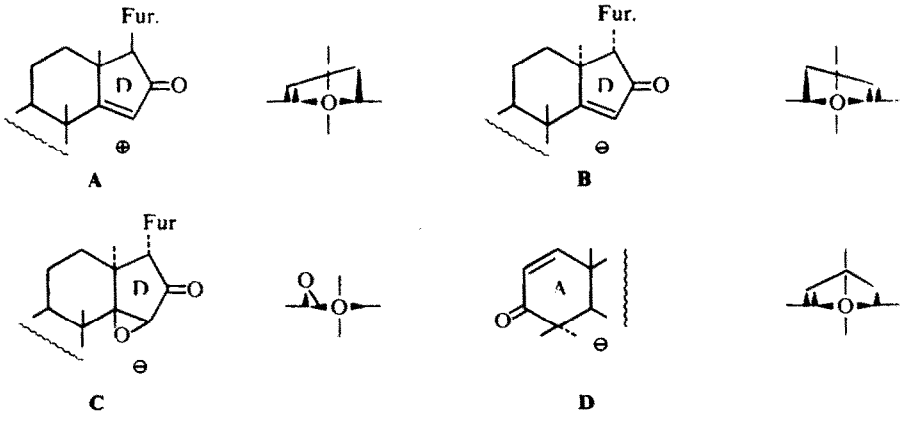


FIG 1. Circular dichroism curves of axadirone (I), axadiradione (II), epoxyazadiradione (III), dihydroazadiradione (V) and dihydroepoxyazadiradione (VIIa)

transition and the sign of the dichroism is determined by the absolute stereochemistry in the vicinity of the chromophore. In 6- and 7-membered rings, the octant rule is "normal", while in cyclopentenones an "inverse" rule has to be considered, i.e. the same chirality gives rise to opposite signs of the circular dichroic bands in the two systems. The circular dichroism curves of the compounds I, II, III, V and VIIa in Fig 1 have been interpreted on the basis of these rules. Any interaction of the furan

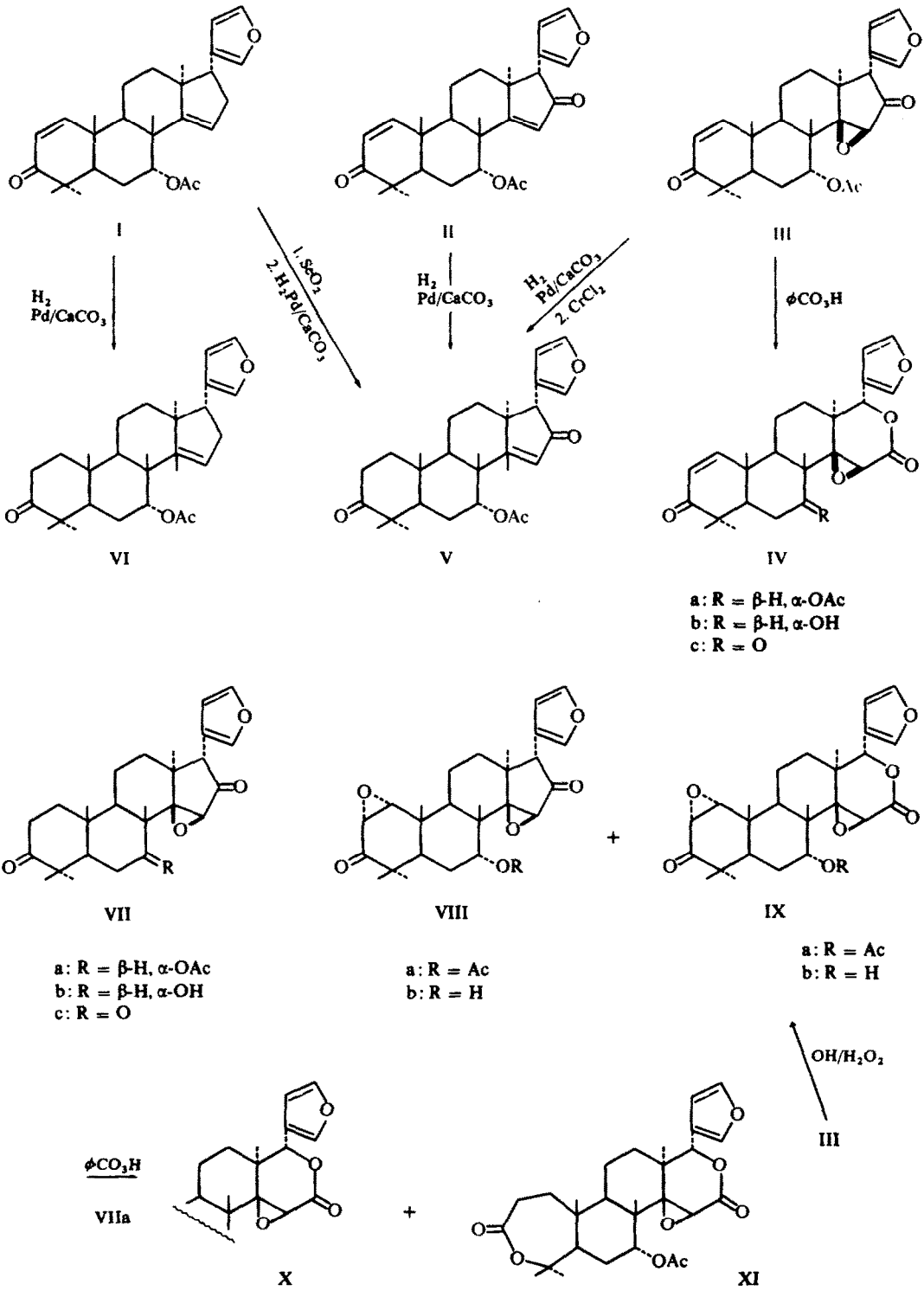


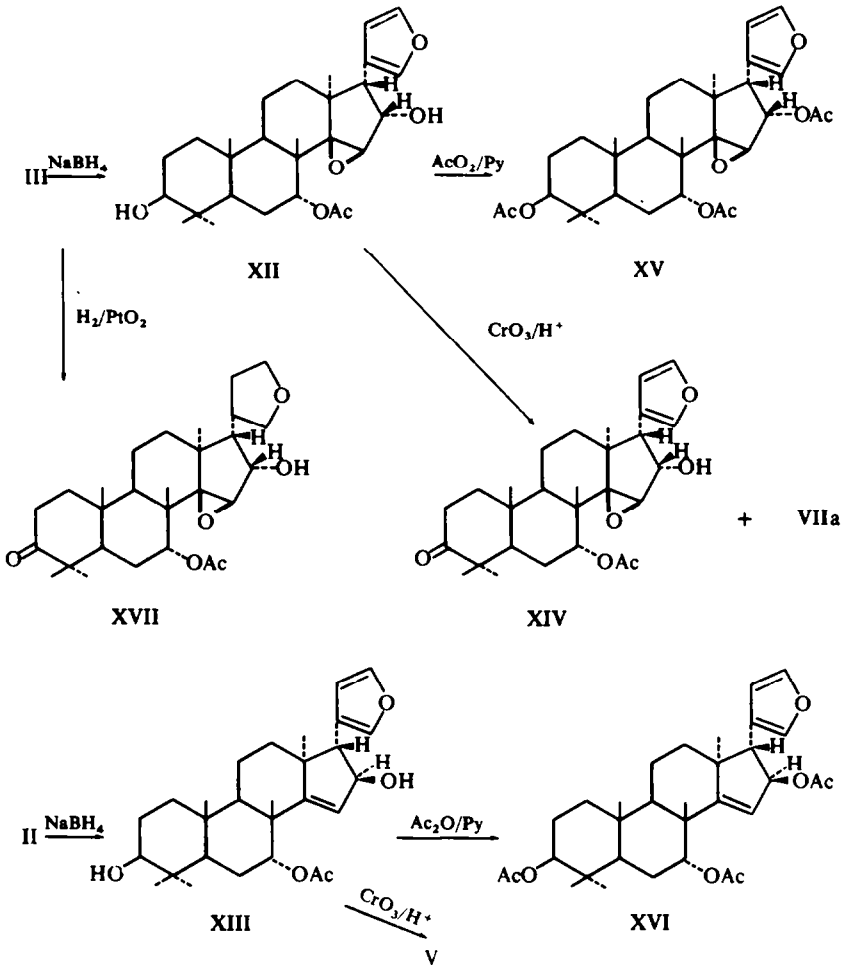
ring with the chromophores has not been taken into consideration since the $\Delta\varepsilon$ values observed are comparable with those for similar "isolated" chromophores. Since chromophore **A** displays a positive Cotton effect, inversion of the stereochemistry at C-13 and C-17 as in **B** should result in an opposite sign.



For measurement, V has been selected, in this compound the previously present $\alpha\beta$-unsaturated ketone in ring A has been reduced leaving only the chromophore in ring D; it shows a broad negative circular dichroic band at 343 nm ($\Delta\varepsilon$ – 0·71) consistent with the inverted stereochemical environment as shown in **B**, and therefore in agreement with the structure assigned for this part of the molecule in II.

The $\alpha\beta$-epoxycyclopentanones obey the "inverse" octant rules, accordingly a negative Cotton effect is anticipated for the chromophore **C**. Indeed, as expected 1,2-dihydro-14β, 15β-epoxyazadiradione (VIIa) exhibits a strong negative band at


I
II
III
H2 Pd/CaCO3
1. SeO2
2. H2Pd/CaCO3
H2 Pd/CaCO3
2. CrCl2
φCO3H
VI
V
IV
a: R = β-H, α-OAc
b: R = β-H, α-OH
c: R = O
VII
VIII
IX
a: R = β-H, α-OAc
b: R = β-H, α-OH
c: R = O
a: R = Ac
b: R = H
a: R = Ac
b: R = H
OH/H2O2
III
φCO3H
VIIa
X
XI



310 nm ($\Delta\varepsilon$, − 4·03). Thus, the circular dichroism curves provide the absolute configuration of ring D in this series of compounds. These results are in good agreement with similar data obtained for grandifolione.[8]

The cyclohexenone chromophore **D** is expected to exhibit a negative Cotton effect according to the "normal" octant rule. Thus, I with a single αβ-unsaturated ketone system in ring A, displays a negative Cotton effect as anticipated from the similarity of the stereochemical environment of ring A with chromophore **D**. Gedunin (IVa), the structure of which has been determined by X-rays,[9] also shows the same Cotton effect.

All the compounds of the meliacin type, having a 5-membered ring D, isolated and characterised till now, have been assumed to possess a β-oriented hydrogen at C-17 on biogenetic grounds. The stereochemistry at C-17 has been now determined by the NMR spectral analysis of the $NaBH_4$ reduction product of III, to which was assigned structure XII. In its NMR spectrum the vinylic 1-H and 2-H signals, as well as the 17-H singlet, originally present in III, are now missing. Instead, the new doublet observed at δ 2·64 (J = 4·5 Hz) was assigned to the 17-H and the doublet at δ

4·37 (J = 4·5 Hz) to the 16-H (coupled with 17-H). The epoxidic 15-H, singlet at δ 3·45, has lost its original sharpness due to a small coupling with the 16 proton. Furthermore, a multiplet at δ 3·19 was assigned to the 3α-H since $NaBH_4$ reduction of αβ-unsaturated ketones have been shown to give the corresponding β-oriented saturated hydroxy derivative in several steroidal systems.[10] The two OH groups in the molecule were also disclosed by the addition of trichloroacetyl isocyanate (TAI)[11] to the NMR sample, the imide proton signals corresponding to the two OH groups now appearing as singlets at δ 8·50 and 8·75. As yet these data do not substantiate the configuration of the OH group at C-16, a problem solved by using the α-orientation of the C-13 Me group determined through the conversion of epoxyazadiradione (III) to gedunin (IVa) *vide infra.*

Pyridine induced solvent shift[12] of the C-13 Me group in compound XII was of a value. $\Delta^{CDCl_3}_{C_5D_5N}$ − 0·38 ppm, to be expected of a 1,3-diaxial arrangement, consequently the configuration of the 16-OH is α-oriented and therefore the 16-H is β. In a similar way compound XIII, the $NaBH_4$ reduction product of II, shows again a 1,4 reduction of the αβ-unsaturated ketone of ring A, however, concerning ring D the vinylic 15-H (previously a sharp singlet) is a narrow doublet at δ 5·41 (J = 1·0 Hz) due to coupling with the 16α-H. In contrast to the value given above for the coupling constant of 17-H in XII (4·5 Hz), the value observed in the case of XIII is J = 8·5 Hz at δ 2·62. Now the 16-H signal, due to coupling with both 17β-H and 15-H is a doublet of doublets at δ 4·46, J = 8·5 and 1·0 Hz, implying an α-orientation for this proton. Indeed, pyridine induced solvent shift involving the 16 OH group did not influence whatsoever the position of the C-13 α Me group, thus confirming the 16β-orientation of this OH. In this case (XIII) the situation is reversed than in XII. Consequently the small coupling value 4·5 Hz has to be related to a small dihedral angle for a 16β-H and 17β-H, while 8·5 Hz has to be related to a large angle, namely 16α-H and 17β-H. It can thus be concluded that since 17-H is β, the furanic side chain is α-oriented. Use was made of pyridine induced solvent shifts in the assignment of the Me groups (Table 2).

TABLE 2. PYRIDINE INDUCED SOLVENT SHIFTS ($\Delta^{CDCl_3}_{C_5D_5N}$) OF METHYL GROUPS IN XII AND XIII (IN PPM)

Compound	4α-Me	4β-Me	8-Me	10-Me	13-Me
XII	−0·36	−0·28	−0·01	−0·06	−0·28
XIII	−0·31	−0·22	−0·11	−0·02	+0·04

From the observed values it is evident that due to the β-orientation of the 16-OH group in compound XIII, the C-8 Me group suffers a stronger shift in pyridine than the same group in compound XII, in which the 16-OH group is α-oriented. However, the values observed for the 4α and 4β Me groups in both are practically unchanged. The vinylic 15-H signal also exhibits a shift in pyridine of − 0·39 ppm in agreement with the value observed for such a proton in allylic alcohols.[12]

Oxidation of compound XII with CrO_3 in pyridine afforded two products which after separation by column chromatography (Silica H) were characterized as the diketone VIIa (upper spot) and the monoketone (3-oxo) XIV, (lower spot) in the

NMR spectrum of which the 3α-H signal originally present had now disappeared. Acetylation of XII yielded the triacetate XV: 3α-H, δ 4·58, 16β-H doublet δ 4·40 (J = 4·5 Hz), and three Me signals for the Ac groups. Similarly XIII yielded the triacetate XVI: for 16α-H doublet of doublets δ 6·10 (J = 8·5 and 1·0 Hz), whereas in this case the 17β-H is shifted to lower field (doublet 2·90 J = 8·5 Hz) due to the interaction with the 16β-OAc group. Oxidation of compound XIII with Jones' reagent afforded exclusively the 1,2-dihydroazadiradione (V).

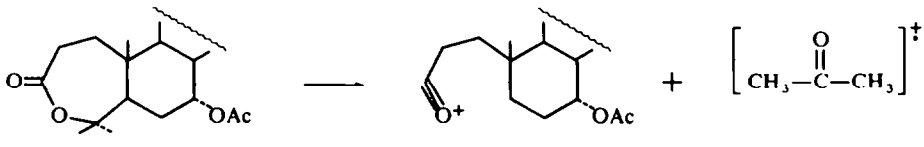
Catalytic hydrogenation of III over PtO_2 for 2½ hr afforded exclusively the hexahydroderivative XVII in which all the double bonds have been reduced as shown by the NMR, UV and IR spectra.

The next step in the oxidative biogenetic degradation sequence referred to in the introduction is the conversion of the epoxyketone ring D in epoxyazadiradione (III) to an epoxylactone as present in gedunin (IVa).[13] To this end III was treated with perbenzoic acid to yield gedunin (IVa) in 90% yield identified by comparison with an authentic sample. Through this experiment III was interrelated with gedunin (IVa) of known structure[9] identifying thereby unequivocally compounds I and II. Interestingly, upon treatment of III with 2N NaOH and H_2O_2 (30%), four products were obtained namely 1α, 2α-epoxy-epoxyazadiradione (VIIIa), 1α, 2α-epoxy-7-deacetoxy-7α-hydroxyepoxyazadiradione (VIIIb), 1α, 2α-epoxygedunin (IXa) and 1α, 2α-epoxy-7α-deacetoxy-7α-hydroxygedunin (IXb), the latter two compounds being identified with authentic samples.

The NMR spectra of the former two compounds VIIIa and VIIIb show a set of two doublets at 3·66 and 3·45 (J = 4·5 Hz) for the epoxidic protons at C-1 and C-2. These signals replace those assigned to the vinylic protons attached to the same C atoms in the starting compound III. That VIIIb is the hydrolysis product of VIIIa was inferred from its NMR spectrum in which the acetate Me signal was not present, while the 7β-H now geminal to the OH group is a triplet at higher field, δ 3·62 (compare to VIIa and VIIb).

Concerning the formation of the 7-membered ring lactones occurring in nature and exemplified by obacunone[3] and nomilin,[4] a similar approach as that of converting a 5-membered ring ketone to a 6-membered ring lactone (ring D) could be used. For such a conversion (from a 6- to a 7-membered ring), 1,2-dihydro-epoxyazadiradione (VIIa) was found to be a perfect substrate. It was anticipated that through a one step reaction such a dual ring expansion could be performed, and indeed upon treatment of VIIa with perbenzoic acid, two products were obtained, namely 1,2-dihydrogedunin (X) (ring D expansion), 1,2-dihydro-7α-obacunyl acetate (XI) (ring A and D expansion).

The structure of XI was confirmed through the analysis of its NMR spectrum, two signals at low field (δ 1·43 and 1·41 for the two 29 and 30 Me groups), the deshielding effect being due to the adjacent ether oxygen of the newly formed 7-membered ring lactone. These positions are in agreement with those observed for the same Me groups in obacunone.[3] Additional evidence for structure XI was obtained from the



fragmentation pattern in its mass spectrum in which an interesting fragment was *m/e* 58 corresponding to the $(CH_3)_2CO$ moiety which could be accounted for only if the ether oxygen of the 7-membered ring lactone is adjacent to the C-4 *gem*-Me groups.

Also the IR spectrum of XI shows a band at 1770 cm^{-1} corresponding to a 7-membered ring lactone.

It is of interest to mention that from *M. azadirachta* L. 7-deacetoxy-7α-hydroxygedunin IVb was also isolated, a compound identical with the alkaline hydrolysis product of gedunin (IVa). Upon oxidation, IVb afforded 7-deacetoxy-7-oxo-gedunin (IVc), of known natural occurrence,[14] which was also shown to be convertible to andirobin.[15, 16] This reaction occurring through a Baeyer Villiger type oxidation now in ring B, forms the ε-lactone, which opens smoothly with toluene-*p*-sulphonic acid in benzene to give the exocyclic double bond at C-8.

EXPERIMENTAL

M.p were taken on a Fisher–Johns apparatus. Optical rotations refer to $CHCl_3$ solns. IR spectra were recorded on a Perkin–Elmer Infracord model 137 spectrophotometer equipped with a NaCl prism, and were determined in KBr pellets. UV spectra were recorded on a Cary 14 in EtOH solns unless otherwise stated. NMR spectra were recorded on a Varian A-60 spectrometer, for 5–10% solns in $CDCl_3$ containing TMS as internal standard M.wts and mass spectra were determined using an Atlas CH4 instrument. Elemental analyses were performed in our analytical laboratory under the direction of Mr. R. Heller.

Isolation procedure. Melia azadirachta L. berries were collected in India during March 1966, sun dried and the oil pressed out, 22% of the dry weight. The oil (2 kg) was suspended in light petroleum and extracted with 80% aqueous MeOH. This soln was then diluted 4 times its volume with water and extracted with $CHCl_3$. The residue obtained after evaporation was chromatographed on a florisil column and eluted with benzene. The eluate after removal of the benzene constituted about 60% of the total $CHCl_3$ extract. This residue was rechromatographed on a silica gel H column in benzene, and the column eluted with successive increase of $CHCl_3$ concentration.

Azadirone (I). The fractions having an R_f 0·5 on TLC (benzene–EtOAc 9:1) were rechromatographed on acid washed alumina (1:40) and eluted with $CHCl_3$–benzene (1:9). The eluate I (350 mg) could not be induced to crystallise; $[\alpha]_D$ +26° (*c* 0·75); λ_{max}^{hexane} 225 nm (ε 10,000); ν_{max} 1740 (acetate), 1680 (cyclohexenone), 1245, 886 (furan) cm^{-1}. (Found: C, 76·8; H, 8·2; M^+ 436; $C_{28}H_{36}O_4$ requires: C, 77·03; H, 8·31%; M.wt. 436·57).

Azadiradione (II). The fractions containing II as the main spot on a chromatoplate were combined and rechromatographed on acid washed alumina. The $CHCl_3$ eluate gave an oil showing one spot on a chromatoplate; $[\alpha]_D$ −24° (*c*, 0·9); λ_{max}^{hexane} 225 nm (ε 14,800); ν_{max} 1739 (acetate), 1710 (cyclopentenone), 1680 (cyclohexenone), 886 (furan) cm^{-1}. (Found: M^+ 450; $C_{28}H_{34}O_5$ requires: M.wt. 450·55).

Epoxyazadiradione (III). The fractions with an R_f 0·37 (benzene:EtOAc 9:1), were combined and crystallised from MeOH to yield III, m.p. 202–203°; $[\alpha]_D$ +4° (*c*, 1·0); λ_{max}^{hexane} 225 (ε 10,800); ν_{max} 1751 (αβ-epoxycyclopentenone), 1736, 1678 and 886 cm^{-1}. (Found: C, 72·1; H, 7·3; M^+ 466; $C_{28}H_{34}O_6$ requires: C, 72·08; H, 7·35%; M.wt. 466·55).

Gedunin (IVa). The fractions with an R_f 0·31 (benzene–EtOAc 9:1), were collected and crystallised from MeOH to yield IVa, m.p. 218°; $[\alpha]_D$ −44° (*c*, 1·1); λ_{max} 225 nm (ε 9000); ν_{max} 1740 (acetate and αβ-epoxy-δ-lactone), 1680, 886 cm^{-1}. (Found: C, 69·6; H, 7·0; M^+ 482; $C_{28}H_{34}O_7$ requires: C, 69·69; H, 7·10%; M.wt. 482·55).

7-Deacetoxy-7α-hydroxygedunin (IVb). The fractions with R_f 0·65 ($CHCl_3$–acetone 9:1) were collected and the residue after evaporation of the solvent *in vacuo* was crystallised from $CHCl_3$–hexane mixture m.p. 259–262°; $[\alpha]_D$ +75° (*c*, 1·3); λ_{max} 225 nm (ε 8400); ν_{max} 3500 (OH), 1745 (αβ-epoxy-δ-lactone), 1680 and 886 cm^{-1}. (Found: C, 70·8; H, 7·3; M^+ 440; $C_{26}H_{32}O_6$ requires: C, 70·89; H, 7·32%; M.wt. 440·52).

Hydrogenation of I *to* VI. Compound I (130 mg) in MeOH (40 ml) was stirred with $Pd/CaCO_3$ (30 mg) under an atmosphere of H_2. The uptake of H_2 was rapid (8 min) and 6 ml H_2 were absorbed. The mixture was filtered through a silica gel (0·05–0·2 mm Merck) plug and the residue crystallised from MeOH (120 mg)

m.p. 106–110°; $[\alpha]_D$ +6° (*c*, 2·0); UV end absorption; ν_{max} 1740, 1710 and 886 cm^{-1}. (Found: C, 76·4; H, 8·5; M^+ 438; $C_{28}H_{38}O_4$ requires: C, 76·67; H, 8·73%; M.wt. 438·58).

Oxidation of I *to* II. (200 mg) in dioxan-water (1:4, 40 ml) was stirred at 60–70° with SeO_2 (300 mg) for 8 hr. The black ppt of Se was filtered through a silica gel plug and the solvent evaporated under reduced pressure (below 50°). The residue was chromatographed on a silica-H column (30 g) and the benzene–$CHCl_3$ (9:1) eluate yielded the oily II (70 mg).

Hydrogenation of II *to* V. Compound II (200 mg) in MeOH (59 ml) was stirred with $Pd/CaCO_3$ at atmospheric press under H_2. After an initial period of induction 11 ml H_2 were absorbed. The mixture was then filtered through a silica gel plug and the solvent removed *in vacuo*. The crude product was then crystallised from acetone–pentane; m.p. 178–179°; $[\alpha]_D$ − 28° (*c*, 0·9); λ_{max}^{hexane} 217 and inflexion at 224 nm (ε 8600); ν_{max} 1710, 1735 and 886 cm^{-1}. (Found: C, 74·2; H, 8·1; $C_{28}H_{36}O_5$ requires: C, 74·30; H, 8·02%).

Hydrogenation of III *to* VIIa. Compound III (950 ml) in MeOH (100 ml) was hydrogenated over $Pd/CaCO_3$ (80 mg) and 0·5 ml of 10% NaOHaq at atmospheric press. The soln was filtered through a silica gel plug and the solvent removed *in vacuo*; the crude product crystallised from MeOH to yield VIIa, m.p. 199–200°; $[\alpha]_D$ 0·0° (*c*, 0·1); λ_{max} 217 nm (ε 5400); ν_{max} 1750, 1730, 1710 and 886 cm^{-1}. (Found: C, 71·5; H, 7·6; M^+ 468; $C_{28}H_{36}O_6$ requires: C, 71·77; H, 7·74%; M.wt. 468·57).

Conversion of VIIa *to* V. Zn dust (2 g) was stirred with Hg_2Cl_2 (0·2 g) in water (5 ml) and conc HCl (0·2 ml) for 20 min. The supernatant liquid was decanted and the amalgamated Zn washed with water and then stirred with AcOH (2·0 ml). HClaq (0·5 ml) and $CrCl_2$ (1 g), in a CO_2 atmosphere. The resulting blue soln was poured into the stirred soln of VIIa (130 mg) in acetone (2 ml), again under CO_2, and stirring was continued at room temp for 6 hr. The mixture was then diluted with water, filtered, washed and evaporated. The residue was chromatographed on silica gel H (Merck) with $CHCl_3$. The fractions with R_f 0·85 ($CHCl_3$–acetone 9:1) were combined, they crystallised from an acetone–pentane mixture to yield V (28 mg); m.p. 178–179°; $[\alpha]_D$ −30° (*c*, 0·6); ν_{max} 1735, 1710 and 886 cm^{-1}; λ_{max}^{hexane} 217 and inflexion at 224 nm (ε 8500). (Found: C, 74·4; H, 8·1; M^+ 452; $C_{28}H_{36}O_5$ requires: C, 74·30 H, 8·02%; M.wt. 452·55).

Hydrogenolysis of III *to* VIIb. III (150 mg) in dioxan (50 ml) and water (3 ml) was hydrogenated with $Pd/CaCO_3$ and ml of 20% NaOH soln for 4 hr. The product crystallised from $CHCl_3$ hexane; m.p. 228–232°; $[\alpha]_D$ +20° (*c*, 1·0); ν_{max} 3500 (hydroxyl), 1750, 1710 and 886 cm^{-1}. (Found: C, 73·0; H, 7·9; M^+ 426; $C_{26}H_{34}O_5$ requires: C, 73·21; H, 8·04%; M.wt. 426·53).

Oxidation of VIIb *to* VIIc. Compound VIIb (50 mg) was stirred with CrO_3 (100 mg) in pyridine soln (5 ml) for 16 hr. After the usual work up the CH_2Cl_2 was dried and evaporated to dryness. The residue was chromatographed on a silica gel H (Merck) column and eluted with $CHCl_3$ yielding pure VIIc, crystallised from acetone–pentane, m.p. 265–267°; $[\alpha]_D$ −84° (*c*, 0·6) UV rising end absorption; ν_{max} 1750, 1710 and 886 cm^{-1}. (Found: C, 73·4; H, 7·5; M^+ 424. $C_{26}H_{32}O_5$ requires: C, 73·56; H, 7·60%; M.wt. 424·52).

Hydrolysis of IVa *to* IVb. Compound IVa (400 mg) was dissolved in MeOH (5 ml) and a soln of NaOH (500 mg) dissolved in MeOH was added. The mixture was kept at room temp for 24 hr, and after acidification extracted with $CHCl_3$. The residue was then chromatographed on a silica gel H. The $CHCl_3$ eluate yielded pure IVb, which crystallised from $CHCl_3$–hexane, m.p. 259–262°; $[\alpha]_D$ +75° (*c*, 0·9); λ_{max} 225 nm (ε 8600); ν_{max} 3500 (hydroxyl), 1745, 1680 and 886 cm^{-1}. (Found: C, 70·7; H, 7·2; M^+ 440. $C_{26}H_{32}O_6$ requires: C, 70·89; H, 7·32%; M.wt. 440·52).

Oxidation of IVb *to* IVc. Compound IVb (100 mg) was stirred with CrO_3 (200 mg) in pyridine (10 ml) at room temp for 15 hr. After the usual work up the crude product was chromatographed on silica gel H and eluted with $CHCl_3$. Evaporation of the solvent to dryness yielded pure IVc, crystallised from MeOH, m.p. 263–264°; $[\alpha]_D$ −50° (*c*, 1·3); λ_{max}^{dioxan} 225 nm (ε 8400); ν_{max} 1745, 1710, 1680 and 886 cm^{-1}. (Found: C, 71·1; H, 6·9; M^+ 438. $C_{26}H_{30}O_6$ requires: C, 71·21; H, 6·90%; M.wt. 438·50).

Conversion of III *to* IVa. Compound III (700 mg) was dissolved in dry benzene (40 ml) to which perbenzoic acid (11 ml) in benzene was added, and the soln left at room temp for 2·5 hr. After the usual work up the the $CHCl_3$ layer was dried and evaporated to dryness. The crude product was then chromatographed on silica gel H and eluted with $CHCl_3$–hexane 6:4 mixture. The fractions with R_f 0·31 (benzene–EtOAc 9:1) were collected and the solvent removed *in vacuo*. The product was then crystallised from MeOH and proved to be IVa, m.p. 218°; no depression upon mixture with an authentic sample; identical NMR, IR and UV spectra.

Reaction of III *with* H_2O_2. To a mixture of III (1 g) in MeOH (200 ml) and 5% NaOHaq (0·5 ml), H_2O_2 30% soln (8 ml) was added dropwise. The reaction was then kept in the refrigerator for 3 days. Following

the usual work up the $CHCl_3$ extract was evaporated to dryness. The product showed four spots on chromatoplate ($CHCl_3$–acetone 9:1). Upon chromatography on a silica gel H column and elution with benzene–$CHCl_3$ (9:1) the following compounds which were identified through their R_f were obtained:

(a) R_f 0·65, compound VIIIa, crystallised from MeOH, m.p. 203–205°; $[\alpha]_D$ +9·5° (*c*, 2·0); λ_{max} 217 nm (ε 4500); ν_{max} 1750, 1736, 1700 and 886 cm^{-1}. (Found: M^+ 482. $C_{28}H_{34}O_7$ requires: M.wt. 482·55).

(b) R_f 0·60, compound IXa, crystallised from MeOH m.p. 218–220°; $[\alpha]_D$ +80° (*c*, 2·0); λ_{max} 211 nm (ε 6000); ν_{max} 1740, 1700 and 886 cm^{-1}. (Found: M^+ 498. $C_{28}H_{34}O_8$ requires: M.wt. 498·55).

(c) R_f 0·42, compound VIIIb, crystallised from a $CHCl_3$–hexane, m.p. 221–223°; $[\alpha]_D$ +11° (*c*, 2·0); λ_{max} 217 nm (ε 5000); ν_{max} 1750, 1700 and 886 cm^{-1}. (Found: M^+ 440. $C_{26}H_{32}O_6$ requires: M.wt. 440·56).

(d) R_f 0·30, compound IXb crystallised from $CHCl_3$–hexane, m.p. 284–286°; λ_{max} 215 nm (ε 5500); ν_{max} 1755, 1700 and 886 cm^{-1}. (Found: M^+ 456. $C_{26}H_{32}O_7$ requires: M.wt. 456·52).

Conversion of VIIa *to* X *and* XI. To a soln of VII (1 g) in dry benzene (100 ml), perbenzoic acid in the same solvent (37 ml, 86 mg/ml) was added, and the solution left overnight at room temp. After the usual work up the crude product showing two spots on a chromatoplate ($CHCl_3$–acetone 9:1) was chromatographed on silica gel-H, and eluted with a $CHCl_3$-hexane(1:1). The pure upper spot 1,2-dihydrogedunin (X) was found to be identical with an authentic sample. Further elution yielded the lower spot, 1,2-dihydro-7α-obacunylacetate (XI), crystallised from $CHCl_3$–hexane, m.p. 197–199°; $[\alpha]_D$ + 2° (*c*, 0·1); λ_{max} 217 nm (ε 8600); ν_{max} 1770 (seven membered ring lactone), 1745 and 886 cm^{-1}. (Found: M^+ 498. $C_{28}H_{34}O_8$ requires: M.wt. 498·55).

$NaBH_4$ *reduction of* III *to* XII. To III (300 mg) dissolved in EtOH (70 ml) $NaBH_4$ (300 mg) was added, and the mixture stirred at room temp for 20 min. After the usual work up the crude product was chromatographed on silica gel (0·05–0·2 mm Merck). Elution with hexane–Et_2O (55:45) yielded pure XII as an oil (180 mg), single spot on a chromatoplate ($CHCl_3$–acetone 9:1) and which could not be induced to crystallise; $[\alpha]_D$ −53° (*c*, 0·1); λ_{max} 217 nm (ε 8000); $\nu_{max}^{CHCl_3}$ 3500, 1735 and 886 cm^{-1}. (Found: M^+ 472. $C_{28}H_{40}O_6$ requires: M.wt. 472·60).

Acetylation of XII *to* XV. To XII (100 mg) dissolved in pyridine (2 ml) Ac_2O (2 ml) was added and the mixture was left overnight at room temp. After the usual work up the pure XV was obtained, crystallised from MeOH, m.p. 128–130°; $[\alpha]_D$ −39° (*c*, 0·1); λ_{max} 217 nm (ε 9000); ν_{max} 1740 and 886 cm^{-1}. (Found: M^+ 556. $C_{32}H_{44}O_8$ requires: M.wt. 556·67).

Oxidation of XII *to* XIV *and* VIIa. To XII (130 mg) in pyridine (16 ml) was added CrO_3 (130 mg) and the mixture stirred at room temp for 36 hr. The crude product showing two spots on a chromatoplate ($CHCl_3$–acetone 9:1) was chromatographed on silica gel. Elution with hexane–Et_2O (7:3) afforded pure VIIa (upper spot); crystals from MeOH found identical with an authentic sample. Further elution with hexane–Et_2O (1:1) yielded the lower spot XIV as an oil which could not be induced to crystallise; $[\alpha]_D$ −93° (*c*, 0·1); λ_{max} 217 nm (ε 8600); $\nu_{max}^{CHCl_3}$ 3500, 1735, 1710 and 886 cm^{-1}. (Found: M^+ 470. $C_{28}H_{38}O_6$ requires: M.wt. 470·58).

Catalytic reduction of III *to* XVII. Compound III (200 mg) in EtOH (100 ml) was hydrogenated over PtO_2 catalyst (60 mg) at atmospheric press for 2½ hr. After the usual work up pure XVII (190 mg) was obtained as an oil which could not be induced to crystallise; UV end absorption at 200 nm; $\nu_{max}^{CHCl_3}$ 3500, 1735 and 1710 cm^{-1}. (Found: M^+ 474. $C_{28}H_{42}O_6$ requires: M.wt. 474·62).

$NaBH_4$ *reduction of* II *to* XIII. To II (1 g) dissolved in MeOH (150 ml) $NaBH_4$ (1 g) was added and the mixture stirred at room temp for 20 min. After the usual work up the crude product was chromatographed on silica gel. Elution with $CHCl_3$–hexane (7:3) yielded pure XIII (upper spot) as an oil which could not be induced to crystallise; $[\alpha]_D$ 0·0° (*c*, 0·1); λ_{max} 217 nm (ε 8400); $\nu_{max}^{CHCl_3}$ 3500, 1735 and 886 cm^{-1}. (Found: M^+ 456. $C_{28}H_{40}O_5$ requires: M.wt. 456·60).

Acetylation of XIII *to* XVI. Acetylation of XIII with Ac_2O in pyridine afforded pure XVI as an oil which could not be induced to crystallise; $[\alpha]_D$ +20° (*c*, 0·1); λ_{max} 217 nm (ε 8800); $\nu_{max}^{CHCl_3}$ 1735 and 886 cm^{-1}. (Found: M^+ 540. $C_{32}H_{44}O_7$ requires: M.wt. 540·67).

Oxidation of XIII *to* V. Compound XIII (50 mg) in acetone (10 ml) was oxidised with Jones reagent. After the usual work up the crude product was chromatographed on silica gel and eluted with $CHCl_3$. Evaporation of the solvent afforded a product identical in all respects with V.

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