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DIASIN, A DITERPENE FROM *CROTON DIASII**

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Key Word Index—*Croton diasii*; Euphorbiaceae; diasin; rearranged labdanic diterpene; structural determination.

Abstract—The trunkwood of *Croton diasii* Pires (Euphorbiaceae) contains diasin, a novel rearranged labdanic diterpene which includes furan, carbomethoxy, γ- and δ-lactone moieties. All ^{13}C NMR signals were assigned and used in conjunction with PMR data, for the elucidation of the relative configuration of all chiral centers. A possible biogenetic pathway to diasin is discussed.

INTRODUCTION

Croton diasii Pires, a new euphorbiacean [2], is an arboreous species which may attain 15 m by 30 cm. The analysed specimen was collected by the botanist João Murça Pires at the bank of igarapé Aurá, on the EMBRAPA property, Belém, Pará State.

RESULTS

Fractionation of the C_6H_6 extract of the trunkwood led to the isolation of sitosterol, ferulates of fatty alcohols and a compound designated diasin. The O atoms of its molecular formula $C_{21}H_{24}O_7$, determined by HRMS, were assigned to γ-lactone (ν_{max} 1700 cm^{-1}) δ-lactone (ν_{max} 1740 cm^{-1}) ester (ν_{max} 1720 cm^{-1}) and furan (ν_{max} 3145, 1500, 870 cm^{-1}) moieties. The furan is β-substituted, as indicated by PMR signals (Table 1) typical of one β (δ 6.42) and two α (δ 7.44) protons. The base peak of the MS (*m/e* 94.0416) most probably is also due to this system linked to a vinyl group (calc. *m/e* 94.0419). In the parent compound, the C_2 group linked to furan is saturated. This fact is revealed by PMR, both the methine and one of the methylene hydrogens being represented by double doublets. The methine signal is easily analysed, since it appears at characteristically low field (δ 5.45). The LIS-reagent $Eu(fod)_3$ was used at 270 MHz to shift at least one of the methylene signals towards the low field end of the band envelope around 2 ppm. These band correlations were confirmed by decoupling experiments. Partial constitution **1**, which fits these data, recognizes also the fact that, with the sole exception of the furan oxygen, all oxy-functions of diasin are part of carboxy groups.

Partial constitution **1** was confirmed by catalytic hydrogenolysis to a hexahydroderivative (partial constitution **2**), a reaction which was accompanied in the PMR spectrum by disappearance of the bands at δ 7.44, 6.42 and 5.45, assigned to the furan and oxymethine protons. In substitution appeared a 4 proton multiplet around 3.7 and a broad band at 6.1 which could be eliminated by addition of D_2O. Corresponding changes in the IR spectrum concerned the disappearance of the furan bands and the appearance of the typical broad CO_2H-band around 3200 cm^{-1}.

Signal multiplicity (*ddd*) of a further oxymethine (δ 4.86) of diasin revealed its vicinality to 3 hydrogens: one (δ 3.1, *d*) on carbon α to a carbonyl and to a fully substituted position and two represented by signals included in the band envelope around 2 ppm. These correlations were again confirmed by decoupling experiments and led to partial constitution **3**. Since the PMR spectrum indicates that diasin contains only one hydrogen on a carbon vicinal to a carbonyl, and this has already been accounted for, the carbons linked to the terminal carbonyl in **3**, as well as in the carbomethoxyl (δ 3.8, *s*) (**4**) of diasin must be quaternary. PMR evidence points additionally to the presence of a quaternary methyl (δ 1.16, *s*) (**5**).

Partial constitutions **1**, **3**, **4** and **5** show a total of 4 carboxyls and 6 quaternary carbons and must thus share one carboxyl and 3 quaternary carbons. Indeed, according to CMR evidence (Table 1), besides the substituted furan position, only 2 quaternary carbons exist in the molecule. The sole combinations of **1**, **3**, **4** and **5** respecting this requirement are **6** and **7** which define relative positions of all atoms of diasin, except for 2 methines and 3 methylenes evidenced by CMR.

The constitution of a rearranged labdanic diterpene can thus be proposed for diasin. The relative configurational details shown in its formula (**8a**) were deduced as follows.

Alkaline hydrolysis of diasin (**8a**) and remethylation of the reaction product led to isodiasin (**8b**). The reaction was accompanied by two PMR changes: δ_{H-7} 4.86 (*ddd*) → 4.86 (*dd*) and δ_{H-8} 3.1 (*d*) → 2.55 (*s*). Absence of H-7 H-8 coupling in **8b** requires the H-C(7)–C(8)-H dihedral angle to be close to 90°, i.e. the *trans*-attachment of the δ-lactone to the decalin system. In consequence, the BC

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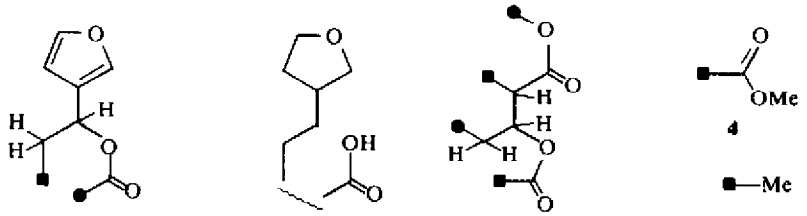
Table 1. NMR data for diasin (**8a**) in $CDCl_3$

Position	Group	^{13}C δ*	^1H δ(100 MHz)	δ(270 MHz)†		J(Hz)
18	CH_3	13.7	1.16	1.42	*s*	
1	CH_2	18.7‡				
2	CH_2	18.6‡				
3	CH_2	29.5		1.7–2.3 (8H)	*m*	
5	CH	50.1	1.5–2.3	2.36 (1H)§	*dd*	13, 4
6	CH_2	24.3		2.52 (1H)‖	*qd*	13, 4
10	CH	42.2		*ca* 2.7 (2H)	*m*	
11	CH_2	42.5				
8	CH	53.7	3.10	3.21	*d*	6
21	CH_3—O	51.9	3.80	3.84	*s*	
7	CH—O	74.9	4.86	4.97	*ddd*	6, 4, 1
12	CH—O	71.3	5.45	5.68	*dd*	12, 4
14	CH	108.1	6.42	6.52	*m*	
15	CH	143.6	7.44	7.44	*m*	
16	CH	139.2	7.44	7.53	*m*	
9	C	36.2				
4	C	45.2				
13	C	124.9				
17	C=O	168.0				
20	C=O	171.1				
19	C=O	177.9				

Notes: * δ(TMS) = $\delta(CDCl_3)$ + 76.9 ppm; † after addition of 0.25 equiv. of $Eu(fod)_3$; ‡ these signals may be interchanged; § H-11eq; ‖ H-1ax or H-2ax



1 2 3 4 5

■ quaternary C
● undefined C

rings of diasin (**8a**) must be *cis*-fused. Epimerization at C-8 of diasin was accompanied furthermore by an IR shift of the δ-lactone carbonyl from 1740 to 1755 cm^{-1}, a phenomenon which is paralleled by the shift observed in the epimerization of columbin (**9a**) into isocolumbin (**9b**) [3]. The furan substituent of the δ-lactone in diasin is equatorial, information derived from the coupling constant which characterizes the H-11 axial, H-12 interaction (J 12 Hz) and shows also the latter hydrogen to be axial.

While the stereochemistry of the lactone is a consequence of the stated facts, configurational analysis of all other chiral centers necessitated assignment and interpretation of CMR data.

The carbon spectrum contains 3 carbonyl peaks at 177.9, 168.0 and 171.1 ppm, assigned respectively to C-19 in analogy to a similar terpenoid [4], the δ-lactone [5–7] and the γ-lactone by default. The 4 additional sp^2 carbon signals can be assigned to the furan moiety, taking into consideration their multiplicity and the shielding caused on carbons 14 and 16 by the β-substituent [5, 8].

Three of the sp^3 carbons, a methyl and 2 methines, are oxygenated. Of the last two, the signal at 71.3 ppm is assigned to C-12 by analogy to similar diterpene furanolactones [8, 9]. The remaining tetrahedral carbons are attributed by signal multiplicity considerations to one methyl, 5 methylenes, 3 methines and 2 quaternary centers. By analogy to other 4-methyl-4-carboxy terpenes, C-4 corresponds to the singlet at 45.2 ppm [4, 10], while the other quaternary carbon, C-9 must thus be represented by the singlet at 36.2 ppm. The third carbon α to a carbonyl group is a methine which can be recognized by its larger residual splitting in the single-frequency off-resonance decoupled (sford) spectrum, with the decoupling frequency set at the high-field end of the proton range. The methylene signal at lowest field can be attributed only to C-11 in view of its sharpness in the sford spectrum (i.e. the absence of second order coupling [11]) and in analogy to a similar system [9]. The remaining 2 methine and 4 methylene signals appear very broadened in the sford spectrum and belong therefore to the strongly proton-coupled chain encompassing carbons 3,2,1,10,5 and 6. Their complete assignment is only possible through the stereochemical arguments that follow.

The configurational problems yet to be solved concern the stereochemistries of C-4 and the AB ring junction. The very high field resonance of the corresponding signal indicates the axial nature of the C-4 methyl group [10]. The chemical shift of C-3 is a good probe of the AB

ring junction stereochemistry. If these two rings were *trans*-fused, the C-3 resonance would appear at *ca* 37 ppm [4, 10]. In diasin (**8a**), however, the lowest-field yet unassigned methylene signal appears at 29.5 ppm, indicating that ring A is axially substituted by C-6 and, therefore, that the two rings are *cis*-fused.

An examination of molecular models indicates that, in the above configuration, ring B of diasin, constrained to a boat form, is flanked by rings A and C, which most probably assume the chair conformation. This being the case, the two bridgehead methines, while having the same number of β effects, are exposed to a different number of γ interactions—one (due to one of the oxygens of the ester function) in the case of C-5, and 3 (due to C-12, C-17 and C-18) in the case of C-10. Thus, the latter carbon absorbs at a higher field. Furthermore, C-6 is axial to ring A, and is shielded by γ interactions with carbons 1 and 3, in addition to C-19. The only two yet unassigned signals are almost coincident and correspond to C-1 and C-2. In *cis*-decalin, the former carbon absorbs at 29.8 ppm [12]. The strong shielding of C-1 in diasin relative to this model reflects the very small C(1)–C(10)–C(9)–C(20) dihedral angle. Due to the reciprocity of this γ effect, carbon 20 is also strongly shielded relative to a typical γ-lactone carbonyl [5]. Finally, the shift of C-2 is very similar in diasin and in 10-methylated diterpenes [10], in spite of the fact that two γ effects act upon C-2 in these models.

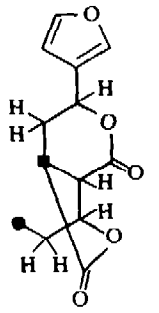
Since only one γ effect is felt by C-2 of diasin, the interaction between carbons 2 and 18 is stronger here than expected, an observation confirmed by the absorption of C-18 at an unusually high-field. In the 10-methylated *trans*-fused diterpenes [10], the action of the same number of γ substituents results in a C-18 shift at 16.1 ppm, as compared to 13.7 ppm in diasin. Taken together CMR data convey the impression that diasin is a highly compact molecule in which strong steric interactions prevail.

Although columbin (**9a**) is an additional example, AB *cis*-fused diterpenoids such as diasin (**8a**) seem to be extremely rare in nature. In the present case, this configuration can be rationalized, considering the rearranged labdanic cation **10** and the derived cyclopropane **11a** and cyclopropanol **11b** as biogenetic intermediates. Indeed, the Euphorbiaceae are known for the presence of hydroxylated cyclopropane derivatives [12]. Opening of cyclopropanols with inversion of configuration is known to occur *in vitro* [14]. Such a reaction would lead to *cis*-fused AB rings and an oxygenated C-19, as observed in diasin (**8a**).

EXPERIMENTAL

Ground wood of *C. diasii* (2 kg) was extracted with C_6H_6 in a Soxhlet apparatus. The extract (30 g) was chromatographed on a silica column. Elution of 40 × 250 ml fractions was performed with C_6H_6 (6 frs), C_6H_6–$CHCl_3$ 4:1 (frs 7–14), C_6H_6–$CHCl_3$ 1:1 (frs 15–28), C_6H_6–EtOAc 4:1 (frs 29–36) and EtOAc (4 frs). The residue of frs 7–14 was rechromatographed to yield ferulates of fatty alcohols (390 mg). The residue of frs 15–28 was washed with petrol and crystallized from MeOH to give sitosterol. The residue of frs 29–36 was rechromatographed by preparative-TLC (Si gel, C_6H_6–EtOAc 1:1) to give diasin (200 mg).

Diasin (**8a**), needles, mp 158–160° (MeOH), $[\alpha]_D^{20}$ + 16° (CH_2Cl_2) (Found: MW 388.1514. $C_{21}H_{24}O_7$ requires: MW 388.1522). ν_{max} (KBr, cm^{-1}): CO bands 1770, 1740, 1720 infl.,



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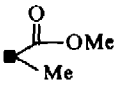

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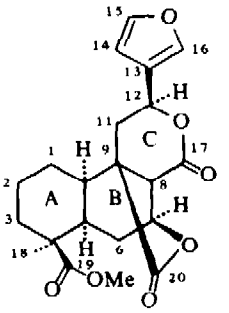
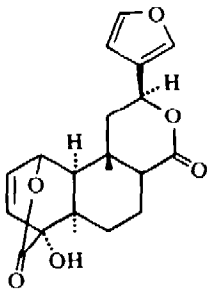
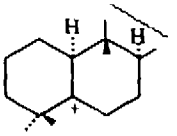


8a βH-8
8b αH-8



9a βH-8
9b αH-8



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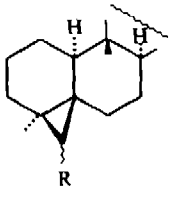

11a R = H
11b R = OH

furan bands 3145, 1500, 870. λ_{max} (CH_2Cl_2, nm): 240 (ε 250). MS, *m/e* (%): 388 (16) M^+, 360 (41), 206 (63), 147 (69), 121 (44), 105 (41), 94 (100), 93 (31), 81 (41), 77 (47), 55 (65), 41 (66).

Isodiasin (**8b**). Diasin (30 mg) in C_5H_5N–H_2O 1:1 (10 ml) containing NaOH (14.3 mg) was left at room temp, for 3 hr. The soln was concentrated *in vacuo*, diluted with $CHCl_3$–Me_2CO 1:1 and acidified with dil HCl. The organic phase was dried, evaporated and the residue treated with excess CH_2N_2 in Et_2O. Evaporation of volatile material gave a residue which was purified by preparative-TLC (Si gel, C_6H_6–EtOAc 3:2) to crystals (22 mg), mp 162–164° (MeOH). ν_{max} (KBr, cm^{-1}): CO bands 1770, 1755, 1715, furan bands 3140, 1500, 870. λ_{max} (CH_2Cl_2, nm): 230 (ε 900). PMR (60 MHz, $CDCl_3$, δ): 7.46 (*m*, H-15, 16), 6.43 (*s*, H-14), 5.46 (*dd*, *J* = 12, 4 Hz, H-12), 4.8 (*d*, *J* = 6 Hz, H-7), 3.8 (*s*, OMe), 2.65 (*s*, H-8), 2.3–1.5 (*m*, H-1,2,3,5, 6,10,11), 1.2 (*s*, Me). MS, *m/e* (%): 388 (15) M^+, 280 (20), 253 (41), 145 (79), 106 (48), 94 (100), 54 (77).

Hexahydrodiasin. Diasin (50 mg) was hydrogenated over 10% Pd/C (50 mg) in dioxan (10 ml) at room temp. and pres. The mixture was filtered and evaporated. The residue was separated by preparative-TLC (Si gel, Et_2O) into 2 products, tetrahydrodiasin (2 mg) and hexahydrodiasin (30 mg), ν_{max} (KBr, cm^{-1}): 3200 br., 1700, 1735, 1710. PMR (60 MHz, $CDCl_3$, δ): 4.83 (*m*, H-7), 3.8 (*s*, OMe), 3.7–3.9 (*m*, 2H-15, 2H-16), 3.1 (*d*, *J* = 6 Hz, H-8), 2.5–1.1 (*m*, 17H), 1.03 (*s*, Me). MS, *m/e* (%): 394 (16) M^+, 348 (10), 295 (13), 277 (41), 205 (23), 144 (100), 131 (33), 119 (31), 105 (42), 91 (48).

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