25. Metabolic Transformation of (3*R*,4*R*)-$\Delta^{1(7)}$-Tetrahydrocannabinol by a Rat Liver Microsomal Preparation[1])

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(15.X.79)

Summary

The metabolism of the non-psychotropic cannabinoid (3*R*,4*R*)-$\Delta^{1(7)}$-tetrahydrocannabinol (**1**) (= $\Delta^{1(7)}$-THC) was investigated in a rat liver microsomal preparation. The metabolites obtained from the incubation mixture were separated, purified and identified by ^{1}H-NMR. spectroscopy and combined gas-liquid chromatography/mass spectrometry. Metabolites **3**–**10** are derived from $\Delta^{1(7)}$-THC (**1**) by monohydroxylation in the isoprenoid moiety or the side chain of the molecule. Metabolites **11**–**16** are hydroxylated in the isoprenoid ring and the side chain simultaneously. The third group, metabolites **18**–**22**, is derived from the 1,7-epoxide **17** by hydrolysis of the oxirane ring, three of these metabolites bearing additional hydroxyl-groups in the isoprenoid part or the side chain. The mass spectra of the metabolites are discussed in detail and a new rule for the fragmentations of tetrahydrocannabinols is presented.

Introduction. – (3*R*,4*R*)-Δ^1-Tetrahydrocannabinol (= Δ^1-THC), the psychotropic principle of hashish, exhibits a large number of pharmacological and biochemical effects [1] [2]. The psychotropic effects of Δ^1-THC may be due to the specific interaction of the drug with a hypothetical THC receptor [3] or to less specific interactions with the lipid phase of the neurolemma by changing its fluidity or permeability [4] [5]. When studying the mechanism of the psychotropic action of Δ^1-THC it is desirable to compare the effects of Δ^1-THC on a particular biochemical system to the effects of a model compound closely resembling Δ^1-THC, but devoid of psychotropic activity. This would allow differentiation between the highly specific interaction of Δ^1-THC with the postulated receptor and other, less specific effects, which could be mimicked by the model compound. The model compounds used so far, cannabinol and cannabidiol, show little similarity to Δ^1-THC. Therefore, based on model considerations, (3*R*,4*R*)-$\Delta^{1(7)}$-tetrahydrocannabinol (**1**) (= $\Delta^{1(7)}$-THC) has been recently proposed by *Binder et al.* [6] as the model compound of choice. $\Delta^{1(7)}$-THC (**1**) closely resembles Δ^1-THC and $\Delta^{1(6)}$-tetrahydrocannabinol

[1]) Presented in part at the 7th ISN meeting, Jerusalem, Sept. 1979.

[2]) Part of the thesis of *U. B.*

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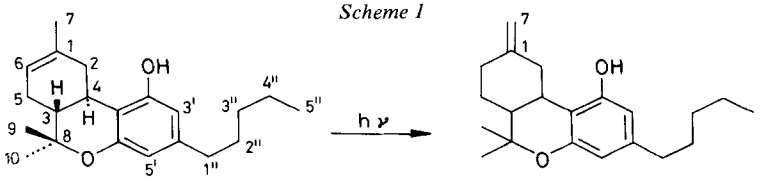
($\Delta^{1(6)}$-THC **2**)[3]) in its physicochemical properties, but slightly differs in its configuration. Measurements on *Dreiding* molecular models, confirmed by NMR. data showed that C(7) of the exocyclic methylidene group is 0.1 nm above the plane of the aromatic ring, while the C(7) of the corresponding methyl group of both Δ^1- and $\Delta^{1(6)}$-THC (**2**) lies 0.2 nm above this plane. $\Delta^{1(7)}$-THC (**1**), tested in the *Rhesus* monkey in dosages up to 5 mg/kg (100× the threshold dose of Δ^1- and $\Delta^{1(6)}$-THC), was devoid of psychotropic activity. This lack of activity may be due to a bad or no fit of $\Delta^{1(7)}$-THC (**1**) to the postulated receptor, based on the difference in stereochemistry, or to the impossibility of the *in vivo* formation of metabolites which in the case of Δ^1- and $\Delta^{1(6)}$-THC (**2**) contribute to the psychotropic activity of the parent compounds, *i.e.* 7-OH-Δ^1-THC, 7-OH-$\Delta^{1(6)}$-THC, 6β-OH-Δ^1-THC and the 3″- and 4″-OH derivatives. Before continuing the investigation of $\Delta^{1(7)}$-THC (**1**) in biochemical systems that have been claimed to be involved in mediating the psychotropic effects of Δ^1- and $\Delta^{1(6)}$-THC (**2**) it was necessary to elucidate the *in vitro* transformation of $\Delta^{1(7)}$-THC (**1**). Because the metabolism of Δ^1- and $\Delta^{1(6)}$-THC (**2**) by rat liver microsomal preparations has been carefully investigated [7] [8] we have chosen the same system for our study on $\Delta^{1(7)}$-THC (**1**). We report on the incubation of **1**, the isolation and structure elucidation of 20 metabolites obtained. Based on these structures, a new rule for mass spectroscopic fragmentation of cannabinoids is presented.

Scheme 1



2 $\Delta^{1(6)}$-THC **1** $\Delta^{1(7)}$-THC

1. Incubation of $\Delta^{1(7)}$-THC (1). – In three similar experiments a total of 410 mg (= 1.3 mmol) **1**, prepared by an improved method [6] *(Scheme 1)* according to *Nilsson et al.* [9] by photoisomerisation of (3*R*,4*R*)-$\Delta^{1(6)}$-THC (**2**), was incubated with an enriched rat liver microsomal preparation [10] from 34 male *Whistar* rats (250–300 g). After 2 h at 37° the incubation mixtures were extracted with ether. The crude extracts were investigated for the formation of metabolites by two-dimensional thin layer chromatography (TLC.) and stored in ethanol in the dark at −20°. The extracts were compared to a blank, prepared by heating an aliquot of the microsomal preparation at 90° for 10 min followed by incubation with **1** under identical conditions. No formation of metabolites was observed.

2. Isolation and purification of the metabolites. – The experimental details of the separation and purification of the metabolites are given in *Scheme 2*.

The extracts were roughly separated in groups of metabolites of similar polarity by batch elution on a *Florisil* column. The mixtures of metabolites thus obtained were further separated by isopolar chromatography on *Sephadex* LH-20 (mono- and dihydroxylated metabolites) and by gradient elution on *Florisil* (di- and trihydroxylated metabolites). The final purifications were achieved by prep. TLC. According to TLC. and gas liquid chromatography (GC.) several metabolites were pure but some still represented mixtures of metabolites.

The monohydroxylated (**3**–**10**) metabolites could be analyzed directly by GC., the di- (**11**–**19**) and trihydroxylated (**20**–**22**) metabolites were converted to their trimethylsilyl ethers prior to GC. in order to increase their volatilily.

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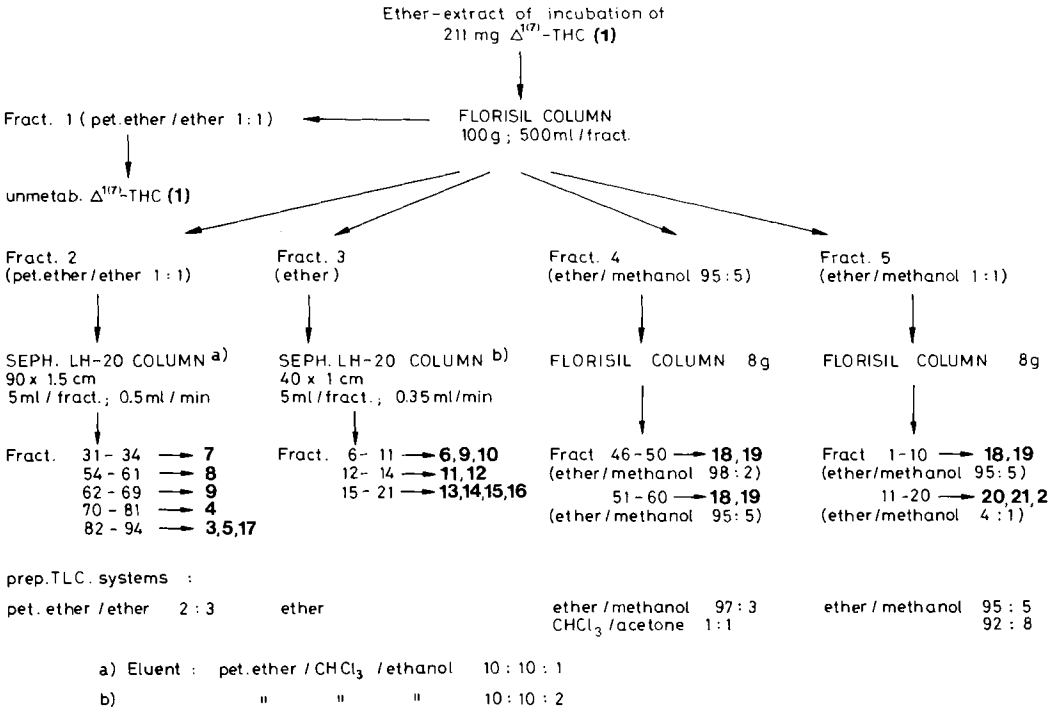
[3]) $\Delta^{1(6)}$-THC (**2**) is not a natural constituent of hashish but a rearrangement product of Δ^1-THC obtained upon storage of the drug. However, **2** closely resembles Δ^1-THC in its psychotropic activity.

Scheme 2. *Isolation of the metabolites*



The yields of the metabolites *(Table 1)* were roughly estimated by comparison of their GC. peak area with reference compounds in known concentration.

Table 1. *Yield of metabolites of* $\Delta^{1(7)}$*-THC* (**1**)

Metabolite	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Yield (μg)[a]	1630	4590	140	1280	760	160	1800	100	70	440
Metabolic conversion in %	0.39	1.12	0.03	0.31	0.18	0.04	0.44	0.024	0.017	0.11
Metabolite	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**
Yield (μg)[a]	480	500	500	200	40	2700		100	100	100
Metabolic conversion in %	0.117	0.12	0.12	0.05	0.009	0.66		0.02	0.02	0.02

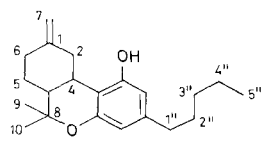
[a]) Obtained from 410 mg of **1**.

3. Structures of the metabolites *(Scheme 3)* were elucidated by NMR. spectroscopy and combined gas liquid chromatography/mass spectrometry[4]) (GC./MS.).

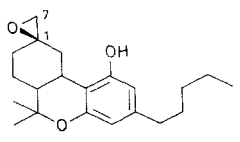
3.1. ^{1}*H-FT-NMR. spectra* were obtained from metabolites **3**, **4**, **7**, **8**, **9** and the mixture of **18** and **19**. The spectra revealed a purity greater than 90% for metabolites **3** and **4** while **7**, **8** and **9** contained considerable amounts of non-cannabinoid

[4]) The authors are greatly indebted to Dr. *W. Dietrich* and Mrs. *I. Dröge*, Dept. of NMR. spectroscopy, Ruhr-University, for recording the NMR. spectra and to Dr. *D. Müller* and Mrs. *Wagner*, Dept. of MS. spectroscopy, Ruhr-University, for recording the mass spectra.

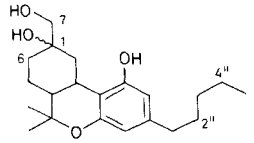
Scheme 3. *Structures of the metabolites*



monohydroxylated metabolites



dihydroxylated metabolites



metabolites derived from the epoxide **17**

3	HO– at C	(6α)	**11**	HO– at C	(2″,6α)	**18**	HO– at C	(1β,7)
4		(6β)	**12**		(2″,6β)	**19**		(1α,7)
5		(2α)	**13**		(3″,6α)	**20**		(1α,6α,7)
6		(2β)	**14**		(4″,6α)	**21**		(1α,2″,7)
7		(2″)	**15**		(4″,6β)	**22**		(1α,4″,7)
8		(3″)	**16**		(4″,2β)			
9		(4″)						
10		(5″)	**17**	1β,7-epoxide				

impurities which allowed a reasonable interpretation only in combination with the MS. data.

The NMR. spectra of cannabinoids and their side chain hydroxylated metabolites have been discussed [11–13] and the ^{1}H-NMR. spectrum of $\Delta^{1(7)}$-THC (**1**) has been analyzed [6]. Decoupling experiments led to the conclusion that the isoprenoid ring of **1** adopts a slightly flattened chair conformation. The ^{1}H-NMR. spectra of metabolites **3**, 6α-hydroxy-$\Delta^{1(7)}$-THC and **4**, 6β-hydroxy-$\Delta^{1(7)}$-THC, give further proof for this conclusion and, therefore, will be discussed in detail.

In metabolite **3** *(Fig. 1)* the broad multiplet of the exocyclic methylidene group H–C(7) of $\Delta^{1(7)}$-THC (**1**) is resolved into two signals centered at $\delta = 4.95$ and 5.05 ppm. The signal of H_β–C(6) appears as a broad multiplet centered at 4.2 ppm, owing to a large *trans*-diaxial coupling to H_α–C(5), a smaller synclinar coupling to H_β–C(5) and additional 1–2 Hz couplings with the protons at C(7).

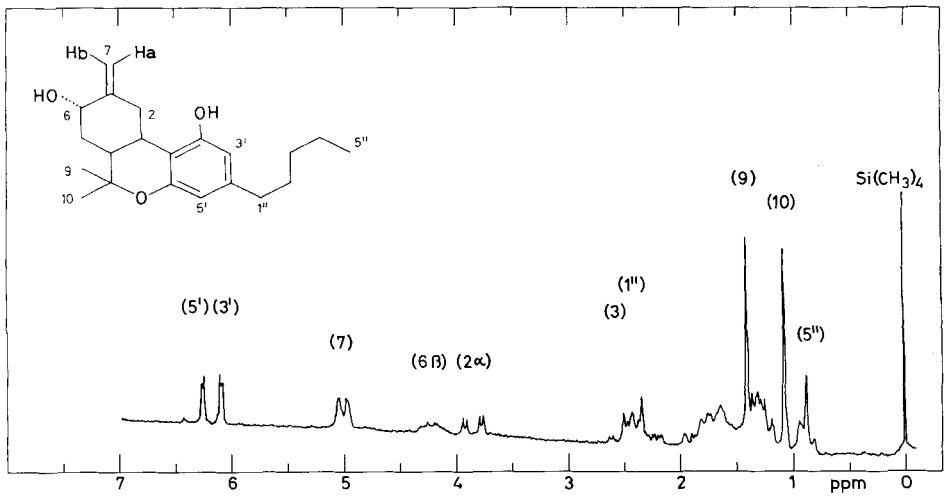


Fig. 1. *90 MHz ^{1}H-FT-NMR. spectrum of 6α-hydroxy-$\Delta^{1(7)}$-THC* (**3**) *in $CDCl_3$*

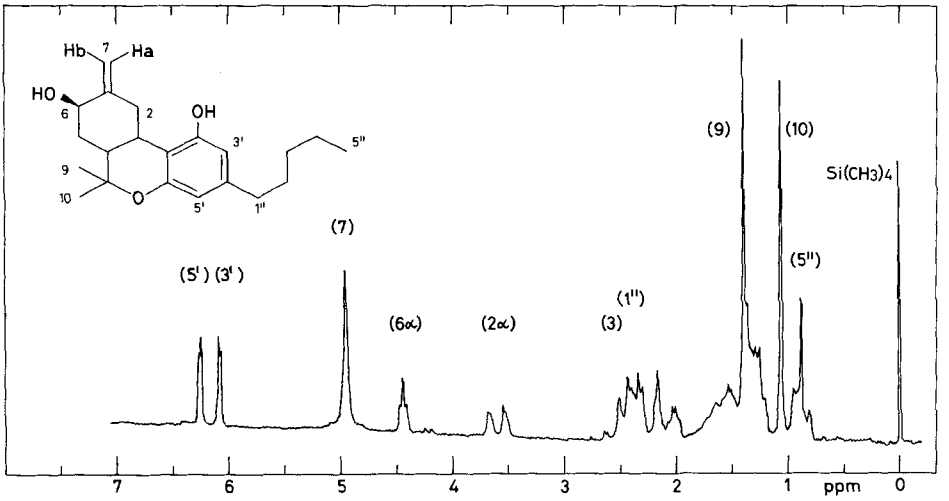
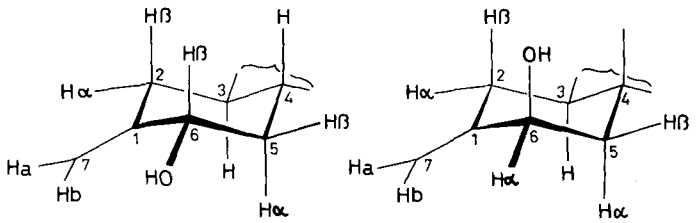


Fig. 2. *90 MHz ^1H-FT-NMR. spectrum of 6β-hydroxy-$\Delta^{1(7)}$-THC (4) in $CDCl_3$*

Since in the spectrum of metabolite **4**, 6β-hydroxy-$\Delta^{1(7)}$-THC *(Fig. 2)*, the signal of the C(7)-protons appears as a 2 H-singlet at 4.97 ppm, the lower field signal of **3** (5.05 ppm) should be assigned to H_b–C(7) slightly deshielded by the equatorial HO-group in 6α-position. The 6α-proton of **4** gives rise to a sharp triplet at 4.44 ppm, actually a doublet of doublets due to equal couplings to the 5α- and 5β-protons of 3 Hz (equal dihedral angles of 60°). The conformations of the isoprenoid rings of **3** and **4** are given in *Scheme 4*, the chemical shifts of the relevant protons of $\Delta^{1(7)}$-THC (**1**), **3** and **4** being listed for comparison.

Scheme 4. *Conformation and ^1H-NMR. data of $\Delta^{1(7)}$-THC (1), 6α- (3) and 6β-hydroxy-$\Delta^{1(7)}$-THC (4)*



3 **4**

Compound	Protons (δ in ppm) 2α	7a	7b	6α	6β
$\Delta^{1(7)}$-THC (**1**)	3.73	4.74			
	d×d	*m*			
6α-hydroxy-$\Delta^{1(7)}$-THC (**3**)	3.83	4.95	5.05		4.2
	d×d	*s*	*s*		*m*
6β-hydroxy-$\Delta^{1(7)}$-THC (**4**)	3.61	4.97		4.44	
	d×d	*s*		*t*	

The presence of the undisturbed signals of protons H–C(2), H–C(1″), H–C(5″), H–C(9), H–C(10), H–C(3) (*cf. Fig. 1* and *2*), the changes in the signals of $H_{a,b}$–C(7) and the splitting patterns of the C(6)-proton clearly indicate, that hydroxylation has occurred in 6*α*-position in metabolite **3** and in 6*β*-position in metabolite **4**. The splitting patterns observed for H_β–C(6) and H_α–C(6) exclude the possibility that the isoprenoid ring might adopt a boat conformation [6]. If this were the case proton 6*β* would be eclipsed by proton 5*β* and H_α–C(6) by H_α–C(5), leading to identical, or, at least very similar signals for H–C(6) in **3** and **4**.

Though metabolites **18** and **19** on column chromatography and on TLC. in several solvent systems behaved like a single compound, the NMR. spectrum revealed the presence of two components. The spectrum showed a single set of signals arising from the protons H–C(3′) and H–C(5′) at the aromatic ring and the side chain protons at C(1″) and C(5″) and a doubling of the signals of the methyl groups C(9) and C(10). A broad singlet at 3.46 ppm arising from the C(7)-protons led to the suggestion that the material was a mixture of the epimeric diols **18** and **19**. This was confirmed by the MS. of these compounds and by the synthesis of the 1*β*, 7-diol.

3.2. Mass spectra. Because of the low volatility of hydroxylated cannabinoids and the fact that little information is gained from the MS. of underivatized cannabinoid metabolites [12], only the MS. of the trimethylsilyl ethers (= TMS ethers[5])) **3a–22a** of metabolites **3–22** were recorded. The fragmentations of cannabinoids have been discussed in general [14] [15] and special attention has been given to the MS. of the TMS ethers of side chain hydroxylated cannabinoids [12] [13].

A set of fragments characteristic for the position of hydroxylation has been established. To facilitate the discussion of the metabolites of $\Delta^{1(7)}$-THC (**1**), we shall briefly review the known ions adding some new ones characteristic for hydroxylation of the isoprenoid part of $\Delta^{1(7)}$-THC (**1**) *(Scheme 5)*.

Fragments at M^+-15, M^+-43, M^+-56, M^+-57 and M^+-68 indicate that parts of the THC-molecule are unchanged; there is no hydroxylation at C(9, 10), C(8, 9, 10), C(2″, 3″, 4″, 5″) and C(1, 2, 5, 6, 7).

Hydroxylation at C(1″) strongly enhances the relative intensity (RI.) of the fragment M^+-57 of the corresponding TMS ether. 2″-TMSO cannabinoids give the typical ion *m/z* 145, resulting from benzylic cleavage between C(1″) and C(2″) with charge retention on the silyl fragment. The same cleavage in the case of 3″-TMSO cannabinoids, but with *McLafferty* rearrangement and charge retention on the aromatic ring yields the fragment M^+-144. These three characteristic ions usually constitute the base peaks (BP.) of the spectra. Less intense but highly indicative is the fragment *m/z* 117 resulting from *α*-cleavage between C(3″) and C(4″) in 4″-TMSO cannabinoids. 5″-TMSO cannabinoids exhibit the ions M^+-144 and M^+-145 simultaneously with low intensity.

7-TMSO-Δ^1-THC and 7-TMSO-$\Delta^{1(6)}$-THC both give the strong fragment M^+-103 resulting from *α*-cleavage between C(1) and C(7).

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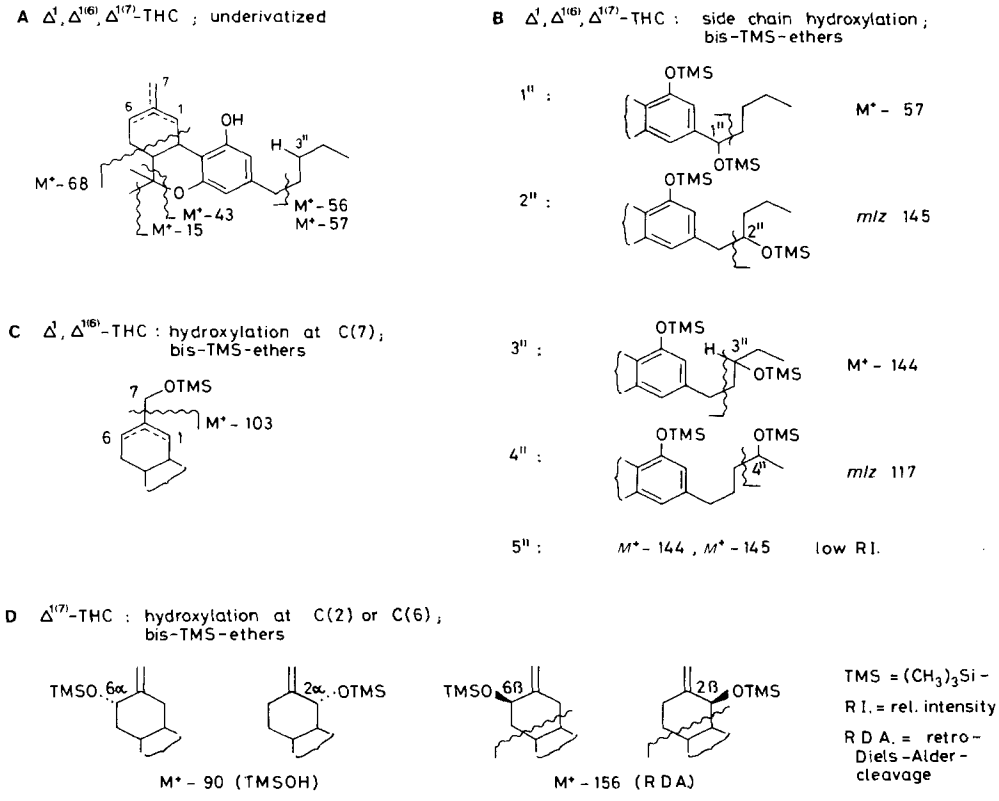
[5]) Though it might lead to some confusion with $(CH_3)_4Si$, the abbreviations TMS for $(CH_3)_3Si$- and TMSO for $(CH_3)_3SiO$- have been generally adopted in the literature and will be used throughout this paper.

Scheme 5. *Characteristic ions and MS. fragmentations of tetrahydrocannabinols*



The following rules concerning hydroxylation of $\Delta^{1(7)}$-THC (**1**) in the 2- and 6-positions are based on the unequivocal identification of 6α-hydroxy-$\Delta^{1(7)}$-THC (**3**) and 6β-hydroxy-$\Delta^{1(7)}$-THC (**4**) by their NMR. spectra. 6α-TMSO-$\Delta^{1(7)}$-THC (TMSO group equatorial) (**3a**) yields a high intensity fragment at M^+-90 (M^+-TMSOH), sometimes in combination with loss of one of the geminal methyl groups C(9) and C(10) as M^+-105, A and A′ respectively. In contrast, in 6β-TMSO-$\Delta^{1(7)}$-THC (**4**) (TMSO group axial) the retro-*Diels-Alder* cleavage (corresponding to M^+-68 in **1** and **2**) is favoured leading to the fragments M^+-156 or M^+-171, B and B′. Usually fragments B and B′ occur in the MS. of metabolites with a 6-α-TMSO group as well, but with low intensity. For compounds with a 6β-TMSO group, A and A′ occur with low intensity. Thus the ratios A:B or A′:B′ can be taken as an indication of whether the TMSO group at C(6) has α- or β-configuration. With 6α-TMSO-$\Delta^{1(7)}$-THC (**3**) the ratios are A:B=40:1 and A′:B′=20:1, with 6β-TMSO-$\Delta^{1(7)}$-THC (**4**) they are 1:5 and 1:3 respectively. Therefore ratios A:B (or A′:B′) >1 indicate α-hydroxylation, ratios <1 β-hydroxylation.

3.3. *The metabolites.* The MS. data of compounds **3a–22a** are summarized in *Table 2*. The molecular ions M^+ are in agreement with the proposed structures; the

Table 2. *MS. data of the metabolites*

Metabolite		M^{+}[a] (RI.)[b]	Fragments (RI.)[c][d]	Ratios A:B	Ratios A′:B′
3a	6α-TMSO-$\Delta^{1(7)}$-THC	474 (5)	$M^{+}-15$ (3), $-$ *90* (39), $-$ *105* (100), -171 (6), -156 (1)	40:1	20:1
4a	6β-TMSO-$\Delta^{1(7)}$-THC	474 (16)	-15 (3), -43 (1), -90 (7), -105 (20), $-$ *156* (20), $-$ *171* (100)	1:3	1:5
5a	2α-TMSO-$\Delta^{1(7)}$-THC	474 (8)	-56 (2), $-$ *90* (6), $-$ *105* (8), -156 (1), -171 (10)	6:1	(1:1)
6a	2β-TMSO-$\Delta^{1(7)}$-THC	474 (43)	-15 (12), -43 (9), -56 (20), -90 (8), -105 (20), $-$ *156* (7), $-$ *171* (60)	(1:1)	1:3
7a	2″-TMSO-$\Delta^{1(7)}$-THC	474 (8)	-15 (8), -90 (1), -105 (1), -144 (8) *m/z 145* (100)		
8a	3″-TMSO-$\Delta^{1(7)}$-THC	474 (5)	-15 (4), -90 (1), -105 (2), $-$ *144* (100)		
9a	4″-TMSO-$\Delta^{1(7)}$-THC	474 (50)	-15 (69), -43 (8), -68 (1), $-68-15$ (11), -90 (6), -144 (17) *m/z 117* (13)		
10a	5″-TMSO-$\Delta^{1(7)}$-THC	474 (16)	-15 (24), -43 (3), $-68-15$ (5) $-$ *144* (12), $-$ *145* (2) *m/z 145* (2)		
11a	2″,6α-bis-TMSO-$\Delta^{1(7)}$-THC	562 (3)	-15 (3), $-$ *90* (13), $-$ *105* (24) *m/z 145* (100)		
12a	2″,6β-bis-TMSO-$\Delta^{1(7)}$-THC	562 (6)	-15 (3), -90 (1), -105 (1) $-$ *156* (3), $-$ *171* (3) *m/z 145* (100)	1:3	1:3
13a	3″,6α-bis-TMSO-$\Delta^{1(7)}$-THC	562 (2)	-15 (2), $-$ *105* (4), -171 (2), $-$ *144* (59), $-$ *144*$-$ *90* (27)		2:1
14a	4″,6α-bis-TMSO-$\Delta^{1(7)}$-THC	562 (5)	-15 (2), $-$ *90* (14), $-$ *105* (45), -156 (5), -171 (27) *m/z 117* (10)	3:1	2:1
15a	4″,6β-bis-TSMO-$\Delta^{1(7)}$-THC	562 (17)	-15 (3), -43 (1), -90 (4), -105 (16), $-$ *156* (11), $-$ *171* (54) *m/z 117* (12)	1:3	1:3
16a	2β,4″-bis-TMSO-$\Delta^{1(7)}$-THC	562 (2)	-90 (2), -105 (6), $-$ *156* (2), $-$ *171* (17) *m/z 117* (17)	1:1	1:3
17a	1β,7-epoxy-HHC	402 (21)	-56 (16), $-56-15$ (9), -73 (10) $-56-43$ (20)		
18a	1β,7-bis-TMSO-HHC	564 (1)	-15 (1), -90 (6), $-$ *103* (20) $-$ *90*$-$ *103* (100)		
19a	1α-HO-7-TMSO-HHC	492 (16)	$-$ *18* (2), $-$ *18*$-$ *103* (100)		
20a	1α,6α,7-tris-TMSO-HHC	580 (1)	-15 (2), $-$ *18* (5), $-$ *18*$-$ *103* (30), $-$ *90*$-$ *18*$-$ *103* (51), $-$ *156*$-$ *18*$-$ *103* (15)	3:1	
21a	1α,2″,7-tris-TMSO-HHC	580 (6)	-15 (2), $-$ *18* (1), $-$ *18*$-$ *103* (17) *m/z 145* (100)		
22a	1α,4″,7-tris-TMSO-HHC	580 (8)	-15 (1), $-$ *18* (1), $-$ *105* (3), $-$ *18*$-$ *103* (64), -144 (9) *m/z 117* (8)		

[a] The molecular weight given by M^{+} always includes an additional TMS group at the phenolic oxygen.
[b] RI. = relative intensity in % of the base peaks.
[c] Fragments are given either as M^{+}- or, when the minor fragment retains the charge, as *m/z*.
[d] When there is no reference to a base peak (RI. = 100%) arising from the cannabinoid, the actual base peak was always the non-specific silyl fragment $Si(CH_3)_3$ at *m/z* 73.

fragment ions are given mainly as differences to M^+, RI. in parentheses, the characteristic ions in italics. In case of 6-hydroxylation, the ratios A:B or A′:B′ are given.

Metabolites **3**, 6α-hydroxy-$\Delta^{1(7)}$-THC and **4**, 6β-hydroxy-$\Delta^{1(7)}$-THC have been discussed under 3.1 and 3.2.

The MS. of metabolites **5a** and **6a** closely resemble those of **3a** and **4a** respectively. The fragments M^+-156 and M^+-171 indicate hydroxylation in the minor retro-*Diels-Alder* fragment. This leaves C(2) and C(5) as the sites of hydroxylation. Since in the rat homoallylic hydroxylation of cannabinoids has not yet been observed and because of the resemblence to the MS. of **3a** and **4a**, we assign the structure of 2α-hydroxy-$\Delta^{1(7)}$-THC to metabolite **5** (A:B = 6:1) and 2β-hydroxy-$\Delta^{1(7)}$-THC to metabolite **6** (A′:B′ = 1:3). Both metabolites differ greatly from **3** and **4** in their TLC. behaviour *(Table 3)*, 2α-hydroxy-$\Delta^{1(7)}$-THC (**5**) being the least polar compound, probably because this metabolite can form an intramolecular hydrogen bond to the phenolic OH-group.

The TMS ethers of metabolites **7**, **8**, **9** and **10** exhibited respectively the characteristic ions *m/z* 145, M^+-144, *m/z* 117 and M^+-144, M^+-145 (low RI.) and thus were assigned the structures of 2″-hydroxy-$\Delta^{1(7)}$-THC (**7**), 3″-hydroxy-$\Delta^{1(7)}$-THC (**8**), 4″-hydroxy-$\Delta^{1(7)}$-THC (**9**) and 5″-hydroxy-$\Delta^{1(7)}$-THC (**10**). The ^{1}H-NMR. spectra of **7**, **8** and **9** could be interpreted based on the MS. data and were in agreement with the assigned structures.

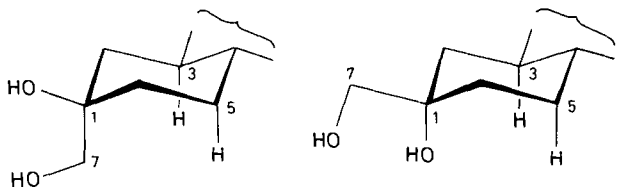
The second set of metabolites **11–16** arises from simultaneous hydroxylation in the side chain and the isoprenoid part of $\Delta^{1(7)}$-THC (**1**). The fragmentations yielding the characteristic ions occur independently from each other, giving a superposition of these fragments *(Table 2)*. The assignments of the structures of 4″,6β-dihydroxy-$\Delta^{1(7)}$-THC (**15**) and 2β,4″-dihydroxy-$\Delta^{1(7)}$-THC (**16**) are based on a comparison of the MS. of the monohydroxylated metabolites **4a** and **6a** and the yields, both **6** and **16** being minor metabolites *(Table 1)*.

The MS. of metabolite **17a** revealed an M^+ peak at *m/z* 402 consistent with the addition of a single oxygen to the $\Delta^{1(7)}$-THC skeleton. Since this oxygen function could not be silylated the structure of 1,7-epoxyhexahydrocannabinol (= 1,7-epoxy-HHC) was assigned to **17**. This is supported by the absence of retro-*Diels-Alder* fragments which require an intact double bond in the isoprenoid ring and the presence of signals at M^+-15, M^+-56, $M^+-56-43$. No second epoxide was detected. Based on model considerations we believe the oxirane ring to have the 1β,7-configuration. There is much less steric hindrance to the attack of an enzyme from the β-face of the molecule than from its α-face. Hydrolytic opening of the epoxide leads to the mixture of diols **18** and **19**, epimeric at C(1).

Upon silylation, the mixture of **18** and **19** yielded two derivatives **18a** and **19a** which could be separated by GC. Independent of the conditions of silylation, the two peaks always appeared in the same ratio. The MS. of the less polar product **18a** gave a molecular ion at *m/z* 562, consistent with a persilylated dihydroxy-hexahydrocannabinol (HHC). Fragments M^+-90, M^+-103 and $M^+-90-103$ (BP.) indicate loss of TMSOH and CH_2OTMS or a combination of both. After initial loss of

TMSOH, the compound behaves exactly like the TMS ether of 7-hydroxy-$\Delta^{1(6)}$-THC. The second product **19a** exhibited a molecular ion at *m/z* 492, corresponding to a monosilylated dihydroxy-HHC (= dihydroxyhexahydrocannabinol). The molecule readily loses water ($M^+ - 18$), then splits off the known fragment of mass 103 to give, the base peak at ($M^+ - 18 - 103$). For reasons of steric hindrance *(Scheme 6)* we conclude the hydroxyl group at C(1) to be in β-position in **18** and in α-position in **19**. It must be assumed that with both diols the primary hydroxyl group at C(7) is silylated first.

Scheme 6. *Configuration of the diols* **18** *and* **19**



In **18** the equatorial hydroxyl group at C(1), being comparatively little hindered, is silylated next, while in **19** no further silylation of the axial hydroxyl group at C(1) is possible owing to the steric hindrance by the bulky TMSO group at C(7), the α-protons at C(3) and C(5), and possibly the axial methyl group at C(8). Likewise *Harvey & Paton* [16] found, that in 1α, 6β-dihydroxy-HHC the 1α-hydroxyl group could not be silylated under mild conditions, *e.g.* using *N, O*-bis(trimethylsilyl)-trifluoroacetamide in acetonitrile. The assignment of the above structures to **18** and **19** was supported by the synthesis of the 1β,7-diol by reaction of $\Delta^{1(7)}$-THC (**1**) with OsO_4 in pyridine. Only one diol was obtained, identical with **18**. Again the synthetic route favours the attack of the large ($OsO_4 \cdot 2$ pyridine) complex from the β-face of the molecule.

The last group of metabolites **20**, **21**, and **22** are derived from the 1α,7-diol **19** by further hydroxylation at C(6α), C(2″) and C(4″) respectively. This is evident from the characteristic ions of their TMS ethers, **20a**, **21a** and **22a**, which show the loss of 18 mass units (H_2O) followed by the cleavage between C(1) and C(7), ($M^+ - 18 - 103$). Metabolite **20a** further loses TMSOH ($M^+ - 18 - 103 - 90$) while the corresponding retro-*Diels-Alder* fragment is completely absent. Compound **21a** exhibits the base peak *m/z* 145 and **22a** gives the typical ion *m/z* 117.

Discussion. – In general the biotransformation of $\Delta^{1(7)}$-THC (**1**) in the rat liver microsomal preparation proceeds in ways similar to those encountered with Δ^1-THC or $\Delta^{1(6)}$-THC (**2**) [7] [8]. There are, however, two major differences. Firstly, as we have demonstrated, the major metabolite of Δ^1-THC and $\Delta^{1(6)}$-THC (**2**), the 7-hydroxy-derivative cannot be formed from $\Delta^{1(7)}$-THC (**1**). Secondly, $\Delta^{1(7)}$-THC (**1**) is metabolized to a much lower extent than the THC's with endocyclic double bonds. Thus our main metabolite (**4**, 1,12% metabolic conversion) was obtained in a yield

20 times lower than 7-hydroxy-Δ^1-THC, the main metabolite of Δ^1-THC [17]. Similarities of the biotransformation of the three tetrahydrocannabinols exist inasmuch as allylic hydroxylations are favoured in the isoprenoid moiety of the cannabinoid and, except for the 1″-position, random hydroxylation occurs in the side chain.

Three distinct groups of metabolites are formed: a) metabolites hydroxylated in positions allylic to the exocyclic double bond (**3**–**6**); b) metabolites hydroxylated in the side chain (**7**–**10**). Both groups of metabolites seem to be suitable substrates for further hydroxylation and metabolites **11**–**16** arise from combined hydroxylation of the isoprenoid part and the side chain of the molecule. c) A distinct group of metabolites **18**–**22** is derived from the epoxide **17**.

Similar epoxides, derived from Δ^1-THC [18] and $\Delta^{1(6)}$-THC (**2**) [16] have been described. The epoxide **17** was obtained in extremely low yield (0.01%) which indicates that the oxirane ring is easily opened to give the mixture of diols or to react covalently with nucleophilic material. The total yield of metabolites derived *via* the epoxy-diol pathway is about 0.6%.

There are probably two types of hydroxylases involved in the transformation of cannabinoids, an 'allylic' hydroxylase which, according to *Bloom & Shull* [19] is also responsible for the epoxide formation and an 'aliphatic' hydroxylase which is responsible for side chain hydroxylation. With $\Delta^{1(7)}$-THC (**1**) the latter favours 2″- and 4″-hydroxylation.

Side chain hydroxylated metabolites of Δ^1-THC and $\Delta^{1(6)}$-THC (**2**) are of considerable psychotropic activity in the *Rhesus* monkey [6] [20] [21]. It may be assumed, that the corresponding metabolites, *i.e.* **7**, **8** and **9**, of $\Delta^{1(7)}$-THC (**1**) have been formed in the *Rhesus* monkey in sufficient amounts to induce behavioural changes after the large *in vivo* dose of 5 mg/kg [6] if they were psychotropically active. This, however, was not the case and it may be concluded, that in fact the exocyclic double bond of $\Delta^{1(7)}$-THC (**1**) prevents psychotropic activity. As in the case of the parent compound **1** this can be understood in terms of drug-receptor interactions, *i.e.* by the inability of **1** and its metabolites to fit to the postulated psychotropic THC-receptor.

Experimental Part

with the collaboration of Mr. *R. Pamp*

General methods: see [22].

Incubation of $\Delta^{1(7)}$-THC (1). – An enriched rat liver microsomal preparation according to *Jones et al.* [10] was used. In a typical experiment the livers of 15 male white *Whistar* rats (250–300 g body weight, pretreated for 4 days with pentobarbital, 12 mg twice a day) were pooled and homogenized in 500 ml ice cold 1.15% aqueous KCl-solution containing 6.5 mmol $MgCl_2 \cdot 2\,H_2O$. The supernatant, obtained after centrifugation for 10 min at 10,000 × g was distributed equally in five 500 ml Erlenmeyer flasks. To each flask was added 20 ml 0.1 mol/l phosphate buffer pH 7.4, 800 mg glucose-6-phosphate, 70 U glucose-6-phosphate dehydrogenase and 160 mg $NADP^+$, followed by 5 ml of an emulsion of **1** prepared by mixing 211 mg **1**, 500 mg Tween 80 and 25 ml 0.1 mol/l phosphate buffer pH 7.4. The mixtures were incubated at 37° for 2 h on a reciprocal shaker, cooled to 5° and extracted with ether (3 × 500 ml). The organic layers were combined, dried (Na_2SO_4), the solvent was evaporated *in vacuo*

at 20° and the extract stored in ethanol at −20° in the dark. No attempt was made to recover acidic metabolites or conjugates.

Separation of the metabolites: see 2 and *Scheme 1.*

Analytical data. - The Rf values of metabolites **3-22**, Δ^1-THC and $\Delta^{1(7)}$-THC (**1**), determined in 3 solvent systems, corrected to Rf (Δ^1-THC)= Rf ($\Delta^{1(7)}$-THC)= 1, are given in *Table 3.*

MS. see *Table 2.*

Table 3. *Rf values of the metabolites*

Compound	Rf		
	[a])	[b])	[c])
Δ^1-THC	1.00	1.00	1.00
$\Delta^{1(7)}$-THC (**1**)	1.00	1.00	1.00
6α-HO-$\Delta^{1(7)}$-THC (**3**)	0.73	0.85	0.70
6β-HO-$\Delta^{1(7)}$-THC (**4**)	0.76	0.85	0.73
2α-HO-$\Delta^{1(7)}$-THC (**5**)	0.86	0.90	0.83
2β-HO-$\Delta^{1(7)}$-THC (**6**)	0.56	0.77	0.57
2″-HO-$\Delta^{1(7)}$-THC (**7**)	0.75	0.87	0.72
3″-HO-$\Delta^{1(7)}$-THC (**8**)	0.63	0.82	0.62
4″-HO-$\Delta^{1(7)}$-THC (**9**)	0.56	0.78	0.57
5″-HO-$\Delta^{1(7)}$-THC (**10**)	0.52	0.77	0.50
2″,6α-di-HO-$\Delta^{1(7)}$-THC (**11**)	0.51	0.72	0.42
2″,6β-di-HO-$\Delta^{1(7)}$-THC (**12**)	0.51	0.72	0.42
3″,6α-di-HO-$\Delta^{1(7)}$-THC (**13**)	0.43	0.67	0.33
4″,6α-di-HO-$\Delta^{1(7)}$-THC (**14**)	0.33	0.62	0.28
4″,6β-di-HO-$\Delta^{1(7)}$-THC (**15**)	0.33	0.62	0.28
2β,4″-di-HO-$\Delta^{1(7)}$-THC (**16**)	0.33	0.62	0.28
1β,7-epoxy-HHC (**17**)	0.86	0.90	0.83
1β,7-di-HO-HHC (**18**)	0.08	0.40	0.12
1α,7-di-HO-HHC (**19**)	0.08	0.40	0.12
1α,6α,7-tri-HO-HHC (**20**)	0.02	0.20	0.03
1α,2″,7-tri-HO-HHC (**21**)	0.02	0.20	0.03
1α,4″,7-tri-HO−HHC (**22**)	0.00	0.15	0.02

[a]) Solvent system: ether.
[b]) $CHCl_3$/acetone 3:2.
[c]) $CHCl_3$/ethyl acetate 3:2.

^{1}H-NMR. spectra (90 MHz, $CDCl_3$, FT): *6α-Hydroxy-$\Delta^{1(7)}$-THC* (**3**): 0.88 (*t*, *J*=6, 3 H, H−C(5″)); 1.08 (*s*, 3 H, H−C(10)); 1.41 (*s*, 3 H, H−C(9)); 2.42 (*t*, *J*=7, 2 H, H−C(1″)); 2.48 (*d*×*t*, *J*=3 and 11.5, 1 H, H−C(3)); 3.83 (*d*×*d*, *J*= −13 and 3, 1 H, Hα−C(2)); 4.2 (*m*, 1 H, Hβ−C(6)); 4.95 (br., *s*, 1 H, Hb−C(7)); 5.05 (br. *s*, 1 H, Ha−C(7)), 6.10 (*d*, *J*=1.5, 1 H, H−C(5′)); 6.25 (*d*, *J*=1.5, 1 H, H−C(3′)).

6β-hydroxy-$\Delta^{1(7)}$-THC (**4**): 0.88 (*t*, *J*=6, 3 H, H−C(5″)); 1.06 (*s*, 3 H, H−C(10)); 1.39 (*s*, 3 H, H−C(9)); 2.4 (*m*, 3 H, H−C(1″), H−C(3)); 3.61 (*d*×*d*, *J*= −12 and 3, 1 H, Hα−C(2)); 4.44 (*d*×*d*, *J*=3 and 3, 1 H, Hα−C(6)); 4.97 (*s*, 2 H, Ha,b−C(7)); 6.10 (*d*, *J*=1.5, 1 H, H−C(5′)); 6.25 (*d*, *J*=1.5, 1 H, H−C(3′)).

1α,7-Dihydroxy-HHC (**18**) and 1β,7-dihydroxy-HHC (**19**): 0.87 (*t*, *J*=5, H−C(5″)); 0.97 (*s*, H−C(10)) and 1.02 (*s*, H−C(10)); 1.25 (*s*, H−C(9)) and 1.34 (*s*, H−C(9)); 2.38 (*t*, H−C(1″)); 3.46 (br. *s*, H−C(7)); 6.13 (*d*, *J*=1.5, H−C(5′)); 6.22 (*d*, *J*=1.5, H−C(3′)).

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