182. Assignment of the 5,6-Double-Bond Configuration in Iloprost and Isoiloprost from ^{13}C-NMR Shifts Determined by 2D Methods

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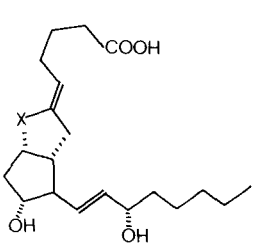
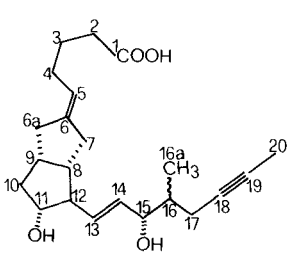
The configurations of the 5,6-double bond in the carbacyclins iloprost (**3**; (*E*)) and isoiloprost (**4**; (*Z*)) are based on a complete assignment of the ^{13}C and ^{1}H resonances determined by 1D and 2D ^{13}C-NMR and ^{1}H-NMR methods.

The therapeutic usefulness of prostacyclin PGI_2 (**1**) [1], a potent vasodilator and inhibitor of blood-platelet aggregation, is limited by the extreme instability of the enol-ether function [2]. The intense search for a stable mimic of prostacyclin was focused on carbacyclin (**2**), in which the cyclic enol-ether O-atom has been replaced by a methylene group [3]. Systematic variation of the longer side chain of carbacyclin (**2**) led to the clinically useful iloprost (ZK 36 374; **3**) [4] with practically the same biological profile and potency as natural PGI_2 [5].

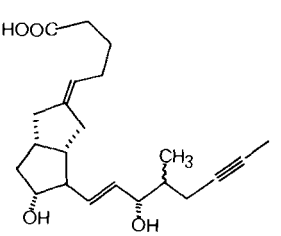
In the synthesis of iloprost (**3**), the shorter side chain is attached to the bicyclo[3.3.0]octanone system by a *Wittig* reaction which gives a separable mixture of (5*E*)- and (5*Z*)-configurated isomers [6]. Up to now, the configuration of the trisubstituted 5,6-double bond was derived by comparison of the biological activities of iloprost (**3**) and isoiloprost (ZK 36 375; **4**); the unnaturally configurated (5*Z*)-isomer **4** displayed markedly lower biological activity.



1 X = O, prostacyclin
2 X = CH_2, carbacyclin



3 iloprost
(ZK 36 374)



4 isoiloprost
(ZK 36 375)

Since we did not want to base the configuration of the 5,6-double bond solely on the biological activity, we tried to obtain an independent structural proof. Our attempts to produce crystals of **3** or **4** or derivatives suitable for X-ray analysis were so far unsuccessful. On the other hand, NMR-spectroscopic methods provide several possibilities to solve this problem. For example, the configuration of the 5,6-double bond in carbacyclin (**2**) was assigned on the basis of nuclear *Overhauser* effects as described by *Kotovych et al.* [7]. Since, however, it is well known that such configurational problems can also be solved by an application of ^{13}C-NMR chemical shifts utilizing the γ-effect [8a], our strategy was focussed on the unambiguous determination of the shielding values of the methylene C-atoms attached to the 5,6-double bond in the two isomers. For this purpose, we made use of 1D and homo- and heteronuclear 2D NMR experiments, which allowed us to achieve a complete correlation of the ^{13}C and ^{1}H chemical shifts in the two diastereoisomers.

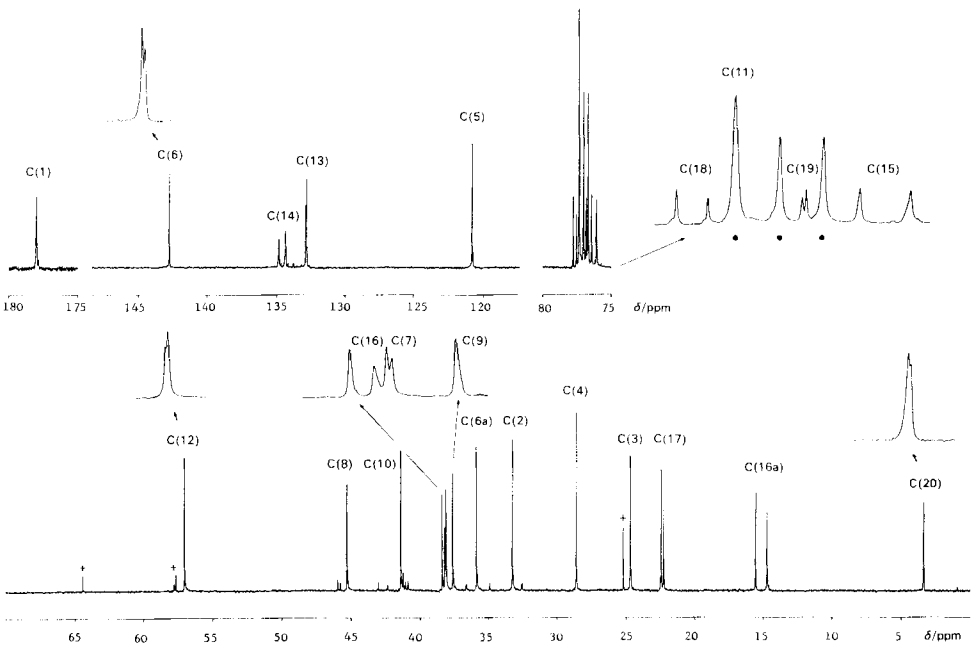


Fig. 1. *^{1}H-Decoupled ^{13}C-NMR spectrum* (100.6 MHz) *of iloprost* (**3**) *in $CDCl_3$*. Expanded insets illustrate the splittings caused by diastereoisomerism originating from C(16). The three dotted signals are due to $CDCl_3$. Nonidentified weaker lines (+) are due to impurities.

Results. – The ^{13}C-NMR spectrum (100.6 MHz) of iloprost (**3**; *Fig. 1*) exhibits a rather complex pattern since many resonances of the 22 C-atoms are split into *d* due to the presence of both C(16) diastereoisomers in a ratio of *ca.* 6:4, as it is evident from the CH_3 resonances. In addition, there are a few weak lines marked by an asterisk which originate from impurities. The doublet splittings are disregarded in the further discussion, however, the values of the individual diastereoisomers are listed in the *Table*, and the magnitude of the splittings supports the signal assignments. A DEPT experiment [9] (θ = 130°) confirms the presence of 2 CH_3, 7 CH_2, and 9 CH groups. Four resonances do

Table. *^{13}C- and ^{1}H-NMR Chemical Shifts of Iloprost* (**3**) *and Isoiloprost* (**4**)

C-Atom	**3**		**4**	
	δ_C[a])	δ_H[b])	δ_C[a])	δ_H[b])
1	177.98	–	177.91	–
6	142.74	–	142.72	–
6	142.72		142.68	
14	134.81	5.48–5.58	134.72	5.45–5.58
14	134.32		134.21	
13	132.86	5.48–5.58	133.01	5.45–5.58
13	132.80		132.93	
5	120.84	5.23	120.90	5.22
5			120.86	
18	77.75	–	77.74	–
18	77.52		77.54	
11	77.32	3.72	77.32	3.68
11			77.26	
19	76.83	–	76.83	–
19	76.80			
15	76.41	4.08	76.52	4.02
15	76.05	3.97	76.10	3.91
12	57.04	1.85	57.73	1.80
12	57.02			
8	45.30	2.05	45.89	2.15
10	41.41	2.32 (β)	40.86	2.30 (β)
		1.17 (α)		1.20 (α)
16	38.41	1.75	38.36	1.70
16	38.24		38.15	
7	38.14	2.35 (β)	32.59	2.10 (β)
7	38.11	2.00 (α)	32.50	2.10 (α)
9	37.64	2.45	36.58	2.45
9			36.51	
6a	35.88	2.35 (β)	41.23	2.45 (β)
		2.05 (α)		2.00 (α)
2	33.26	2.35	33.19	2.35
4	28.56	2.10	28.51	2.10
4		2.00	28.49	2.00
3	24.69	1.70	24.72	1.70
17	22.47	2.25	22.57	2.25
		2.20		2.15
17	22.29	2.00	22.33	2.00
		1.95		
16a	15.57	1.02	15.63	1.03
16a	14.74	0.96	14.72	0.94
20	3.39	1.80	3.42	1.80
20	3.38		3.39	

[a]) δ_C from 1D ^{13}C-NMR spectra rel. to $CDCl_3$ (= 77.00 ppm).

[b]) δ_H rel. to $CHCl_3$ (=7.27 ppm) from 1D ^{1}H-NMR spectra (±0.01 ppm) or from 2D ^{13}C,^{1}H hetereonuclear shift correlated and COSY spectra (±0.05 ppm). The assignments of the α- and β-protons of CH_2(6a), CH_2(7), and CH_2(10) were made according to [7]. Mutual assignments of δ_H and δ_C in the C(16) diastereoisomers of **3** and **4** are not implied.

not appear in the DEPT spectrum and, therefore, originate from quaternary C-atoms. Tentative assignments are possible on the basis of the chemical shifts and effects due to diastereoisomerism originating from C(16), which are only large in the immediate vicinity of C(16) (*e.g.* C(14), C(13) and C(18), C(19)) and decay with increasing distance. The diagnostically important signals of C(6a) and C(7) are located in the range from 22 to 42 ppm which contains the 7 CH_2 resonances. It was, therefore, decided to determine the chemical shifts of these C-atoms by means of 2D $^1H,^{13}C$ relayed coherence transfer experiments [10]. A convenient starting point for such an analysis would be the resonance of C(12) at 57.0 ppm which is well separated from the other aliphatic C-atoms due to the three α-substituents C(13), C(11), and C(8).

In the relayed coherence transfer experiment, magnetization is transferred in two steps. First, ^1H-magnetization transfer occurs among scalar-coupled ^1H-spins (mainly *via* $^3J(H,H)$) followed by further transfer *via* $^1J(C,H)$ onto the directly bonded C-atom. When the ^{13}C-resonance frequencies are detected under broad-band ^1H decoupling, they appear modulated by the frequencies of the 'directly' bonded protons ($^1J(C,H)$) and 'indirectly' bonded protons ($^2J(C,H)$). Thus, it becomes feasible to make ^{13}C- and ^1H-resonance assignments along a C–C chain. A corresponding 2D experiment was carried out for the ^1H-shift range of 0.67–2.67 ppm and a ^{13}C-shift range of 2–80 ppm, and a contour plot is illustrated in *Fig. 2*. Some important correlation peaks are labelled, and the sequence-analysis procedure is performed as follows. The well separated C(12) resonance

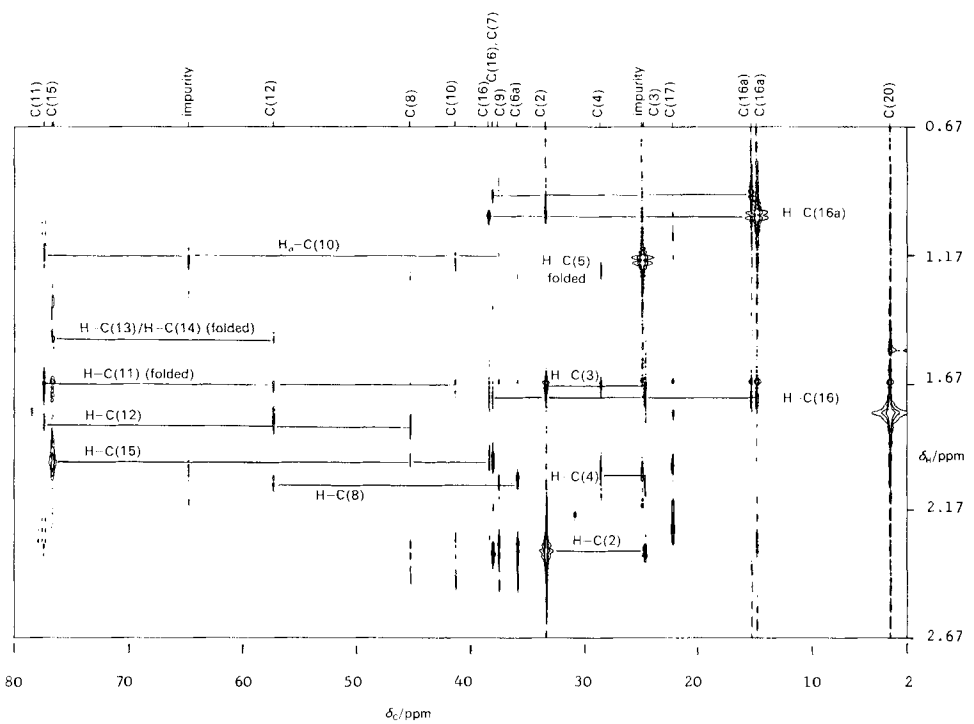


Fig. 2. *2D $^{13}C,^1H$ relayed coherence transfer NMR spectrum of iloprost* (**3**), *contour plot of the total $^{13}C/^1H$ spectral widths.* For details see *Exper. Part.*

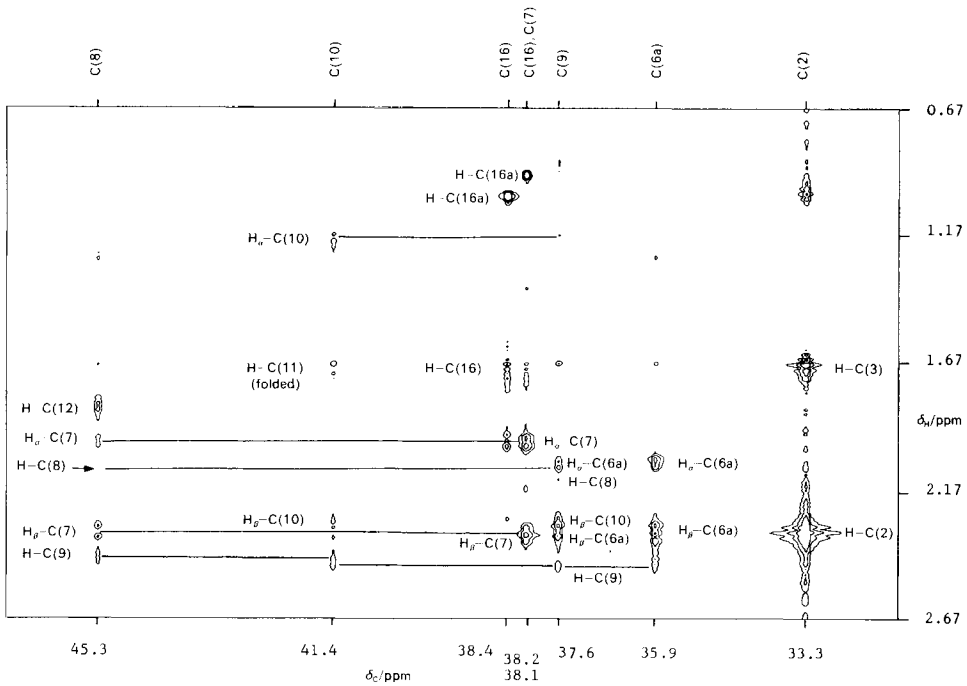


Fig. 3. *Expanded contour plot of the ^{13}C-NMR chemical shift range 31.8–46.8 ppm of the 2D experiment illustrated in* Fig. 2

(57.0 ppm) shows a strong correlation peak at *ca.* 1.8 ppm (H–C(12)) which agrees with the location of this resonance from an ^{1}H,^{1}H-decoupling experiment in which the vinyl protons H–C(13)/H–C(14) were irradiated. The C(12) resonance contains further cross peaks at 2.05 ppm (H–C(8)) and two folded signals originating from ^{1}H resonances at 3.7 ppm (H–C(11)) and 5.5 ppm (H–C(13)). An H–C(12) cross peak appears also in the cross peaks of a ^{13}C resonance at 45.30 ppm which can thus be assigned to C(8) (the C(11) and C(13) resonances appearing at much higher δ values). The H–C(8) peak, too weak to be observable in this contour plot, is clearly visible at 2.05 ppm in the cross section of C(8) through the 2D spectrum. Continuing from the C(8) signal, its vicinal neighbors C(9) and C(7) can be clearly assigned *(Fig. 3)*. In this expanded contour plot of the 31.8–46.8 ppm ^{13}C-shift region, C(8) shows strong cross peaks at 1.85 ppm (H–C(12)), 2.45, 2.35, and 2.0 ppm. The 2.45-ppm cross peak correlates with corresponding ones at 41.4, 37.6, and 35.9 ppm, which are assigned to C(10), C(9), and C(6a), respectively. The C(9) resonance (37.6 ppm) exhibits strong cross peaks at 2.05 and 2.35 ppm which correlate with corresponding peaks for the ^{13}C signal at 35.9 ppm and are attributed to the diastereotopic methylene protons H_α–C(6a) and H_β–C(6a), respectively. Hence, the C(6a) resonance is located at 35.9 ppm. On the other hand, the 2.0- and 2.35-ppm cross peaks of C(8) correlate with strong contours at 38.1 ppm (C(7)) and can be assigned to the diastereotopic methylene protons H_α–C(7) and H_β–C(7). The third methylene group (C(10)) can be located at 41.4 ppm starting from C(8) or C(9) *via* the common cross peak at 2.45 ppm (H–C(9)). C(10) shows cross peaks at 1.2 and 2.3 ppm for the directly bonded protons H_α and H_β,

respectively. The highly shielded H_α–C(10) is typical for the carbacyclins and is well resolved in the ^{1}H-NMR spectra of iloprost (**3**) and the carboprostacyclin analyzed by *Kotovych et al.* (1.15 ppm) [7]. The assignment of the C(10) resonance is further supported by a (folded) cross peak at 3.7 ppm (H–C(11)) which correlates with a strong 'direct' cross peak at 77.3 ppm (C(11)) *(Fig. 2)*. In this way, the resonance positions of the crucial C-atoms C(6a) and C(7) and of the corresponding protons are clearly established. The chemical-shift data are collected in the *Table*.

Similar analyses in other regions lead to a complete assignment of the ^{13}C-NMR spectrum of iloprost. For example, C(11) can be assigned to the resonance of 77.3 ppm (hidden by one of the $CDCl_3$ peaks in the 1D spectrum) since the 2D spectrum shows cross peaks at 3.7 (folded; H–C(11)), 1.2 (H_α–C(10)), 2.3 (H_β–C(10)), and 1.8 ppm (H–C(12)). Another set of correlations is obtained starting from the resonance at 28.6 ppm (C(4)) which gives cross peaks at 5.2 ppm (folded; H–C(5)), 2.05 (H–C(4)), and 1.7 ppm (H–C(3)). The ^{13}C signal at 24.7 ppm (C(3)) shows cross peaks at 1.7 (H–C(3)), 2.05 (H–C(4)), and 2.35 ppm (H–C(2)), whereas the third methylene group (C(2)) of the shorter side chain must be assigned to the signal at 33.3 ppm since it gives a very strong 'direct' cross peak at 2.35 ppm (H–C(2)) and the expected H–C(3) cross peak at 1.7 ppm. Finally, the ^{13}C resonances of the longer (C_9) side chain can be attributed starting from the readily assignable C(16a) methyl resonances (14.74; 15.57 ppm). Both show cross peaks at 1.75 ppm (H–C(16)) besides strong direct peaks for H–C(16a) at 1.02 and 0.93 ppm,

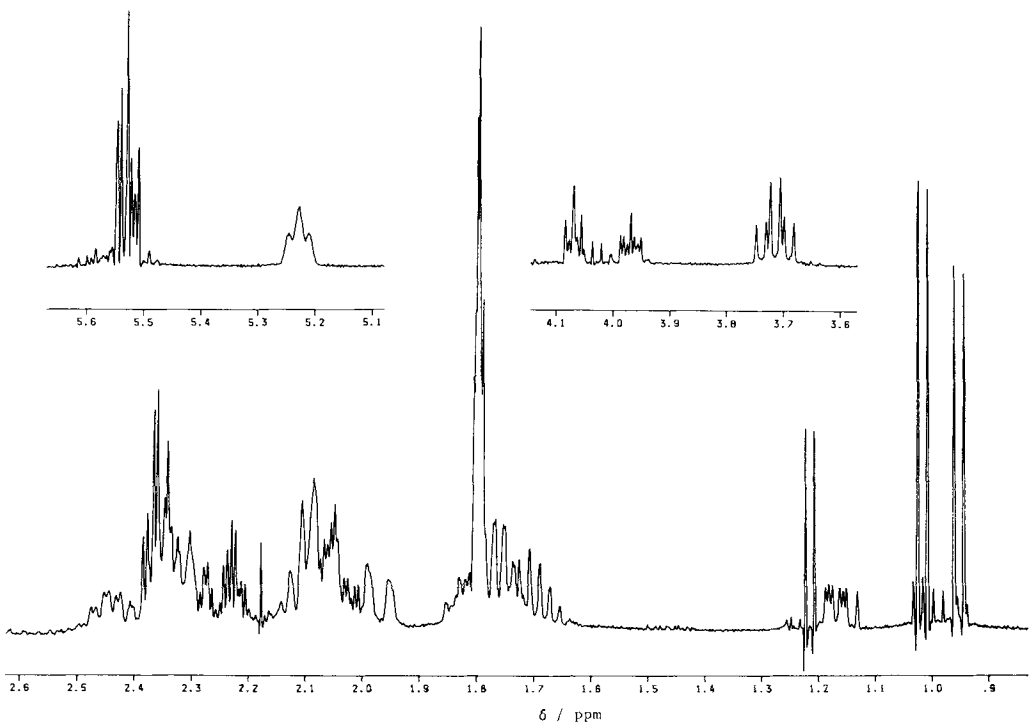


Fig. 4. *^{1}H-NMR spectrum (400 MHz) of iloprost* (**3**) *in $CDCl_3$.* The signals at 1.22 and 4.03 ppm originate from an impurity.

respectively, of the two C(16) diastereoisomers. These allow identification of the C(16) resonances at 38.41 and 38.24 ppm, respectively. They, in turn, lead to the assignment of C(15) (76.41; 76.05 ppm) and C(17) (22.47; 22.29 ppm) *via* common cross peaks.

The ^{1}H-NMR spectrum (400 MHz) of iloprost (**3**; *Fig. 4*) exhibits well separated signals for the 3 vinyl protons centered at 5.53 ppm (H–C(13), H–C(14) and 5.23 ppm (H–C(5)), for the H-atoms geminal to the secondary alcohol functions (H–C(15) at 4.08 and 3.97 ppm and H–C(11) at 3.72 ppm), for H_α–C(10) (1.17 ppm), and for CH_3(20) (1.80 ppm) and CH_3(16a) (1.02; 0.96 ppm). The majority of the ^{1}H signals, *i.e.* of 7 CH_2 and 4 CH groups, appear in the very complex pattern between 1.6 and 2.5 ppm. A homonuclear 2D COSY experiment (300 MHz, *Fig. 5*) together with the results from the heteronuclear 2D relayed coherence transfer spectrum (*Fig. 2* and *3*), however, permits a

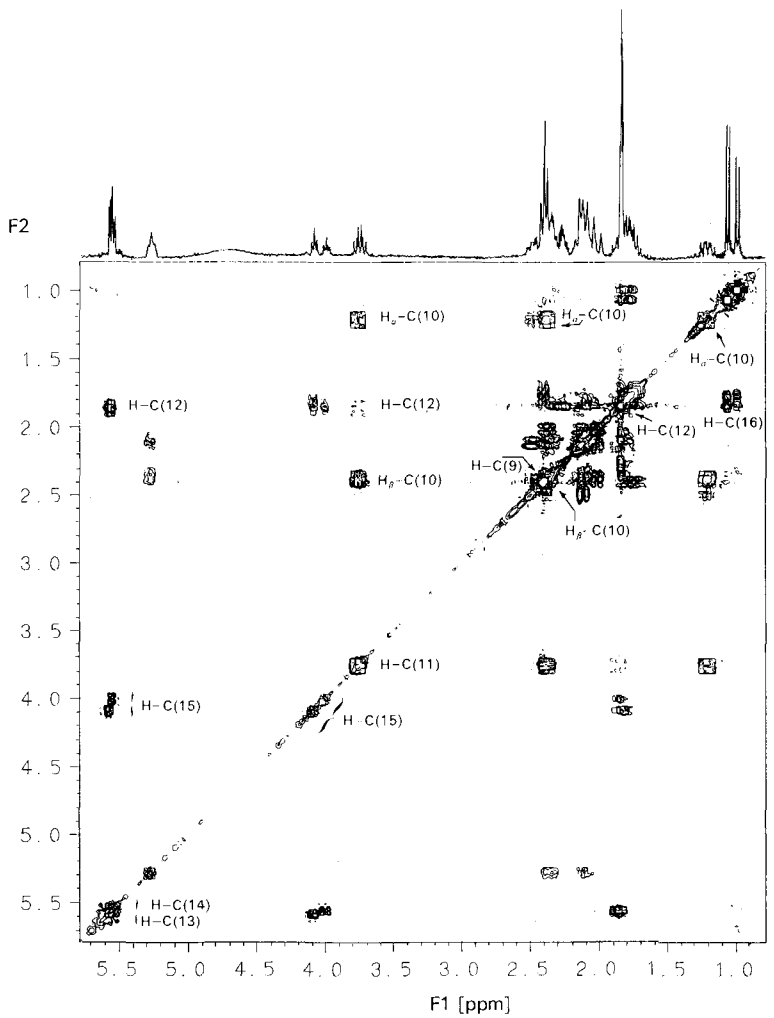


Fig. 5. *^{1}H,^{1}H shift-correlated 2D NMR spectrum (COSY) of iloprost* (**3**). For details see *Exper. Part.*

complete determination of all ^1H shifts as listed in the *Table*. The total plot of the COSY spectrum *(Fig. 5)* confirms the assignments of H–C(15) in the two diastereoisomers *via* twin cross peaks to the vinyl protons and shows the correlations between the H–C(11) signal and the diastereotopic methylene protons H_α–C(10) (1.18 ppm) and H_β–C(10) (2.34 ppm) as well as the other vicinal neighbor H–C(12) (1.82 ppm) which, in turn, correlates with H–C(13). Further, H_α–C(10) is spin-coupled to a resonance at 2.46 ppm, a *q* with *d* fine structure attributed to H–C(9). These ^1H assignments from the COSY spectrum are in excellent agreement with those derived from the 2D $^{13}C,^1H$ relayed coherence transfer spectrum discussed above.

Thirteen protons of **3** form a very complex area of correlations between 1.6 and 2.5 ppm, and assignments are further complicated by the presence of two diastereoisomers. The chemical shifts of these protons (at C(2), C(3), C(4), C(6a), C(7), C(8), and C(17)) are more easily and precisely extracted from the 2D $^{13}C,^1H$ relayed coherence transfer spectrum as described above and presented in the *Table*.

The same strategy for the determination of the ^{13}C and ^1H chemical shifts was applied in the case of the isomeric compound isoiloprost (**4**). The results obtained from a thorough analysis of the 1D ^{13}C- and ^1H-NMR spectra and 2D $^{13}C,^1H$ relayed coherence transfer experiments are summarized in the *Table*. In addition, a normal 2D $^{13}C,^1H$ shift correlation experiment was carried out to complete the $^{13}C,^1H$ correlations *via* $^1J(C,H)$. As expected, the chemical shifts of the C-atoms and protons are very similar in both isomers. The only characteristic differences in the ^{13}C-NMR spectra are found in the region of 32–46 ppm, and they involve the chemical shifts of C(6a), C(7), C(8), and C(9). *Fig. 6* illustrates these results which are of prime importance for the assignment of the 5,6-double-bond configuration in iloprost (**3**) and isoiloprost (**4**).

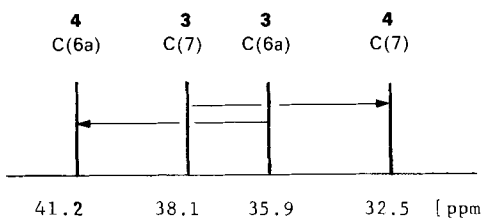
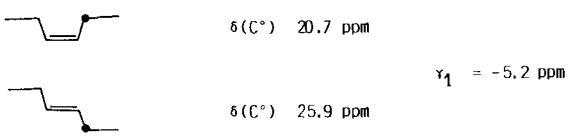


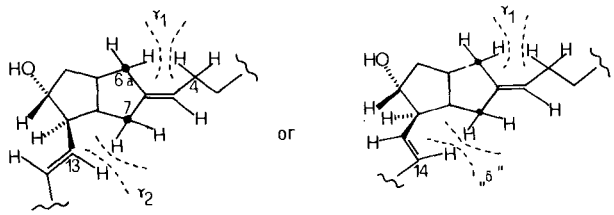
Fig. 6. *Schematic representation of ^{13}C-NMR chemical shifts of C(6a) and C(7) in iloprost* (**3**) *and isoiloprost* (**4**)

Discussion. – The crucial finding that, in going from **3** to **4**, C(6a) is shifted by +5.3 ppm to *higher* frequencies, whereas C(7) suffers a *low*-frequency shift of −5.6 ppm, is the result of a typical γ effect as exemplified in the (*E*/*Z*) pair of 3-hexene [8b]:



The different chemical shifts of C(6a) and C(7) in **3** and **4**, however, are not only determined by the γ-effect originating from the C(4) methylene group, since $|\Delta\delta^7_{6a}|$ is 2.2

ppm for **3** and 8.7 ppm for **4**. Inspection of stereomodels of the skeleton of **3** reveals that the CH(13) or CH(14) groups of the C_9 side chain will exert a second γ (or a δ) shielding effect on the C(7) resonance. For this effect, the mutual interaction of the equatorial CH_3 groups in *trans*-1,2-dimethylcyclohexane may be taken as a model which leads to a shielding effect of −2.6 ppm [11]. Under the assumption that the differences in the



^{13}C-NMR chemical shifts of C(6a) and C(7) in the two isomers are caused solely by the γ_1 and γ_2 effects of the two side chains, the $\Delta\delta^7_{6a}$ values for the two stereoisomers are estimated to be: $\Delta\delta^7_{6a} = -2.6 - (-5.2) = +2.6$ ppm for the (*E*)-isomer (found: +2.2 ppm for iloprost (**3**)) and $\Delta\delta^7_{6a} = (-2.6 - 5.2) - 0 = -7.8$ ppm for the (*Z*)-isomer (found: −8.7 ppm for isoiloprost (**4**)). Therefore, it can be safely concluded both from the relative chemical shifts of C(6a) and C(7) in iloprost and isoiloprost, respectively, and from the respective changes within each stereoisomer that iloprost has the (*E*)-configuration **3** and isoiloprost the (*Z*)-configuration **4**. We believe that the method employed to derive the above results may be of general use in the class of carbacyclins.

Experimental Part

The ^{1}H-NMR spectra were measured at 400.13 MHz on a *Bruker AM-400 WB* spectrometer in 5-mm sample tubes at 24° with 0.05M solns. in $CDCl_3$. ^{13}C-NMR spectra were obtained at 100.6 MHz in 10-mm sample tubes at 34° on the same instrument with 0.2M and 0.25M $CDCl_3$ solutions for iloprost (**3**) and isoiloprost (**4**), respectively.

The 2D-COSY experiments were performed on a *Varian XL-300* spectrometer with a 0.01–0.02M solution of **3** in $CDCl_3$. Free induction decays of 512 × 1024 points were accumulated, each an average of 4 scans. In the processing, the t_1-FID's of 512 points were zero-filled to 1024 points to make up the final data matrix of 1024 × 1024 points in the time domain. A 'pseudo-gaussian' window processing function was employed to provide resolution enhancement.

The 2D ^{13}C,^{1}H relayed coherence transfer spectra were taken at 100.6/400.13 MHz and 22° with a ^{13}C spectral width of 7812.5 Hz (77.65 ppm) and a ^{1}H spectral width of 800 Hz (2.0 ppm). A total of 128 t_1 time increments were used to measure FID's of 2048 points, and 2D-FT was performed without digital filtering after doubling the number of points in t_1 by zero-filling. Sample concentrations for these experiments were 1.25M for **3** and 1.0M for **4**. Therefore, the ^{13}C and ^{1}H chemical shift data of the 2D spectra differ slightly from the data extracted from the 1D spectra obtained with dilute solutions.

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