Tetrahedron Letters No. 48, pp 4735 - 4738, 1973. Pergamon Press. Printed in Great Britain.

CARBON-13 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY OF C/D CIS-POLYOXYPREGNANES. III.[1] STRUCTURE OF 12β-O-CINNAMOYL-20-O-ACETYLGLYCOSARCOSTIN

Takashi Yamagishi, Koji Hayashi and Hiroshi Mitsuhashi*

(Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Hokkaido 060, Japan)

and

Mamoru Imanari and Kazuhiro Matsushita

(JEOL AID Division Application Laboratory, Nakagami 1418, Akishima, Tokyo 196, Japan)

(Received in Japan 21 August 1973; received in UK for publication 10 October 1973)

The structure of a number of polyoxypregnane derivatives from the rhizome of Cynanchum caudatum Max. was reported previously.[2] Further survey of the most polar fraction of the aglycone resulted in the isolation of a diester, a highly oxygenated pregnane derivative (I). In the present communication, we wish to describe the structure of I by the ^{13}C NMR, the ^{1}H NMR, the gas-liquid chromatographic (GLC), and the mass spectral method.

Compound (I), mp 218-221°. $[\alpha]_D^{16}$ +47.6° (c=0,42, MeOH), gave analytical values corresponding to $C_{28}H_{46}O_8$. The IR and UV absorptions [$\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 3000, 1705, 1640, 1590, 1500; λ_{max}^{MeOH} nm (ε): 217 (16.000), 223 (14.000), 278 (23.000)] demonstrated the presence of a trans-cinnamoyl group, while its ^{1}H NMR spectrum showed an OAc signal at δ 1.95 (3H, singlet). Furthermore, the ^{13}C NMR spectrum provided evidence for the presence of the cinnamoyl and the acetyl groups by signals at δ 21.59, 120.73, 124.49, 128.56, 129.35, 130.44, 136.14 and 167.21 which were assigned by a comparison with those of cinnamic acid and acetic acid. Alkaline hydrolysis of I with 5% methanolic KOH for 3 hr gave, two acids and a neutral substance which is termed glycosarcostin (II). These acids were identified as cinnamic acid and acetic acid by GLC.[3,4] Glycosarcostin (II), mp 268-275°, $[\alpha]_D^{20}$ +24.3° (c=0.7, MeOH), is considered to be a pregnane derivative, since its molecular formula was established as $C_{21}H_{36}O_8$, and since its ^{1}H NMR showed the presence of three Me groups at δ 1.47 (3H, doublet), 1.57 (3H, singlet), and 1.80

(3H, singlet). The ^{13}C NMR spectrum of II revealed the presence of eight carbinyl carbons at δ 67.14, 71.51, 72.97, 75.43, 77.86, 78.59, 88.62, and 88.50. Further, the four signals at δ 67.14, 72.97, 77.86, and 71.51 were assignable to tertiary carbons and the other resonances were due to quaternary carbons by off-resonance decoupling experiments. The OH groups may be located at C-3β, C-5α, C-6β, C-8β, C-12β, C-14β, C-17β, and C-20 in II from the biogenetic analogy to other natural polyoxypregnanes from the same plant. These assumptions were confirmed the following results.

The ^{13}C NMR spectrum of II is similar to that of glycolineolon (III) without the resonances of C-12 and the D-ring carbons (Fig. 1). This presumption is supported by the characteristic mass fragmentation of II and its deuterated compound (IIa)[5] (Fig. 2). The structure of II including its stereochemistry was deduced as formula II. This structure was confirmed by the glycolation of IV to II by Fieser's procedure. To the author's knowledge, glycosarcostin is the most oxygenated steroid found in nature. Correlation of the ^{13}C chemical shifts for I and II indicates the following results. The resonances of C-12 and C-20 of I are shifted by about 3 ppm downfield than those of II. On the other hand, the C-11, C-15, C-16, and C-21 signals are shifted upfield, and thus, each acid may be located at either C-12 or C-20 of II.

The location of ester linkage was established by glycolation of penupogenin[7] to V with m-chloroperbenzoic acid followed by treatment with 0.1N H_2SO_4. The final structure of I must be given as 12-O-cinnamoyl-20-O-acetylglycosarcostin.

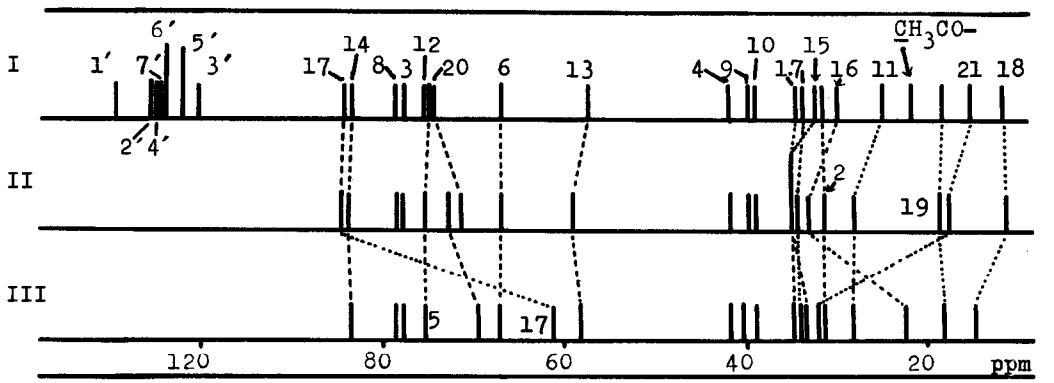


Fig. 1. Correlation of ^{13}C chemical shifts for I, II, and III[8]

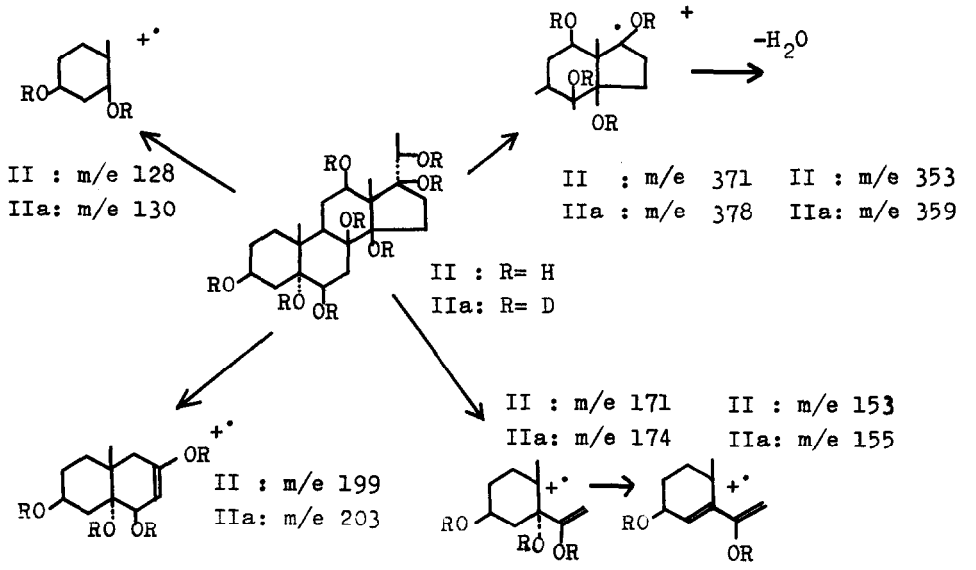
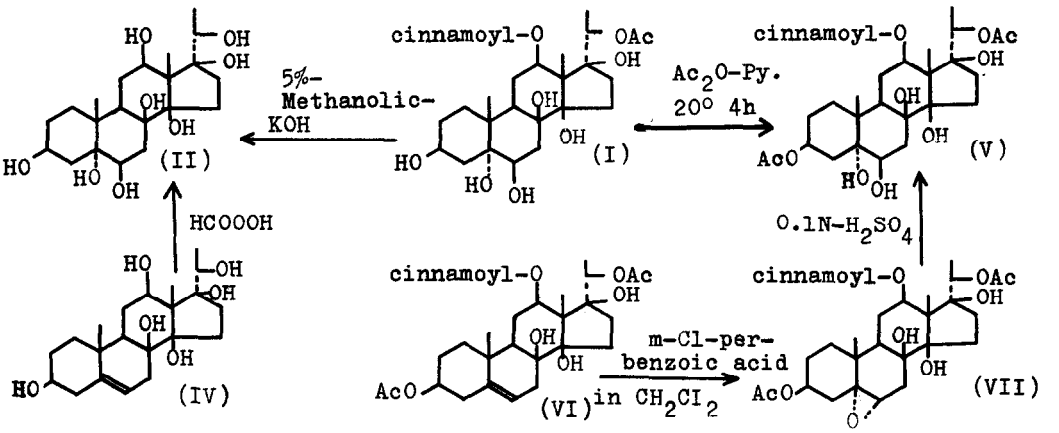


Fig. 2. Principal mass fragmentations of II and IIa



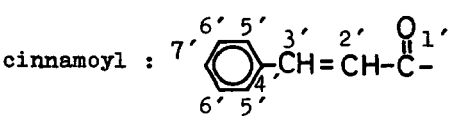


Fig. 3.

REFERENCES

1 Part II: T. Yamagishi, K. Hayashi and H. Mitsuhashi, Tetrahedron Lett. in submittion.

2 Y. Shimizu and H. Mitsuhashi, Tetrahedron 24, 4143 (1968); T. Yamagishi and H. Mitsuhashi, Chem. Pharm. Bull. (Tokyo) 20, 625 (1972); T. Yamagishi and H. Mitsuhashi, ibid. 20, 2070 (1972); T. Yamagishi, K. Hayashi and H. Mitsuhashi, ibid. 20, 2289 (1972); T. Yamagishi, K. Hayashi and H. Mitsuhashi, Tetrahedron Lett. 199 (1972).

3 Cinnamic acid was identified as its methyl ester, Rt. 3.2 min 5% XE-60, 2 m × 3 mm o.d. glass column, column and oven temperature 130°, injection port temperature 250°, detector temperature 250°, FID detector, carrier gas N_2.

4 25% DEGS. 2 m × 3 mm o.d. glass column, column and oven temperature 90°, injection port temperature 250°, detector temperature 250°, FID detector, carrier gas N_2, Rt. 6 min.

5 T. Yamagishi, K. Hayashi and H. Mitsuhashi, Tetrahedron Lett. 4005 (1972); M. Fukuoka, K. Hayashi and H. Mitsuhashi, Chem. Pharm. Bull. (Tokyo) 19, 1469 (1971).

6 L.F. Fieser and S. Rajagopalan, J. Amer. Chem. Soc. 71, 3938 (1949).

7 T. Sasaki, K. Hayashi and H. Mitsuhashi, Chem. Pharm. Bull. (Tokyo) 20, 628 (1972).

8 I. II, and III were measured in pyridine-d_5 . All of the ^{13}C-FT-NMR spectra were obtained at 25.1 MHz using a JEOL PS-100/PFT-100 spectrometer system, and facilitated by the complete proton decoupling technique. The data recorded are in ppm downfield from the ^{13}C resonance of tetramethylsilane.