Communications to the editor

UTILIZATION OF ^{13}C-^{13}C COUPLING IN STRUCTURAL AND BIOSYNTHETIC STUDIES. XI.[1] BIOSYNTHETIC STUDIES OF COARCTATIN

Sir:

Coarctatin (**I**)[2] is a metabolite of the fungus *Chaetomium coarctatum*. Notwithstanding its structural similarity to radicinin, which is formed by the condensation of only acetic acid molecules,[3] a somewhat different biosynthetic pathway (four acetic units plus two C_1 units) for the main framework of **I** has been proposed by TURNER *et al.*[2] However, another pathway involving, for example, the incorporation of propionic acid molecules into C-9, 6 and 5, and C-10, 4 and 3, may operate in the formation of **I**. A recent report on the incorporation of propionic acid into a fungal metabolite aurovertin[4] prompted us to investigate the biosynthesis of **I** by ^{13}C-nmr spectroscopy.

The required ^{13}C assignments for **I** was made as follows and the chemical shifts are listed in Table 1. The assignments of protonated resonances due to C-2, 7, 8 and 9 were obtained easily based on splitting patterns in the off-resonance spectrum, chemical shifts and selective proton decoupling experiments. In view of the difference of solvents used*, the result is in agreement with that reported by TURNER *et al.*[2] However, the assignments of the remaining quaternary sp^2 carbons were not so straightforward. Of these, four at 169.9, 164.3, 163.5 and 162.6 ppm** were assigned to sp^2 carbons bearing oxygen and therefore, the rest two at 99.2 and 119.8 ppm were due to those unsubstituted by oxygen.

Table 1. ^{13}C-Nmr chemical shifts and coupling constants of coarctatin.

Carbon	δc(ppm)[b]	Jc-c(Hz)
8 q[a]	14.6	42
11 q	57.6	
9 t	64.9	
2 d	89.3	80
4 s	99.2	67
6 s	119.8	63
7 d	138.7	42
10 s	162.6	
5 s	163.5	64
1 s	164.3	81
3 s	169.9	67

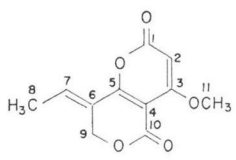
Determined on a JEOL FX-100 spectrometer at 25.05 MHz spectral width; 5 KHz, pulse angle; ~70°, data points; 16 K.

[a] multiplicity of off-resonance decoupling. s; singlet, d; doublet, t; triplet, q; quartet.

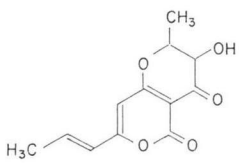
[b] ppm downfield from internal TMS.

In the α-pyron system with oxygen substituents at β- and δ-positions, carbonyl carbons and the oxygenated ones absorb at the same region in the ^{13}C-nmr spectra.[2,5] Therefore, the differentiation of these carbons from each other was made by long range selective proton decoupling (LSPD) experiments[6] and by use of ^{13}C-^{13}C coupling patterns observed in **I** labeled with $^{13}CH_3{}^{13}COONa$.

Irradiation at H-2(δ_H 5.95) collapsed a signal at 164.3 ppm to a sharp singlet which appeared as a broad peak in the proton coupled spectrum. Likewise, the unresolved resonance at 169.9 ppm was perturbed by the irradiation at CH_3O (δ_H 4.10). Thus, these signals were assigned to C-1 and C-3, respectively. Saturation of H-9 (δ_H 5.25) led to an inconclusive result, since it eliminated long range couplings from both the signals at 162.6 and 163.5 ppm. In the ^{13}C-nmr spectrum of $^{13}CH_3{}^{13}COONa$ enriched **I**, (Fig. 1) two pairs



Coarctatin (I)

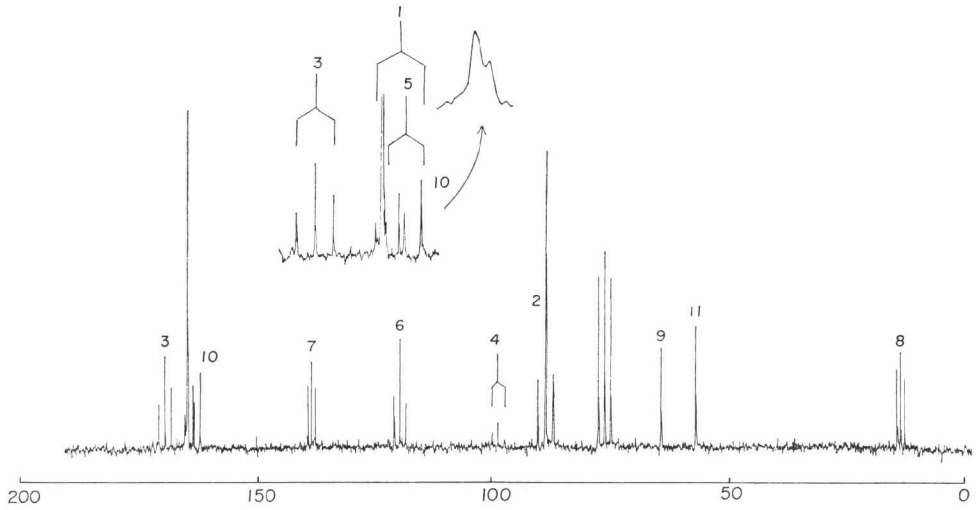


Radicinin

* In the structural studies on **I**, a mixed solvent ($CDCl_3$ supplemented with CF_3CO_2H) was used for the measurements of ^{13}C-nmr spectra.[2] In our experiments, however, CF_3CO_2H was replaced by CCl_3CO_2H (CCl_3CO_2H - $CDCl_3$, 1 : 1) to eliminate strong quartet peaks due to CF_3CO_2H.

** ^{13}C-Nmr spectra were obtained as reported previously[1] and the chemical shifts are expressed in ppm from internal TMS.

Fig. 1. Proton noise-decoupled ^{13}C-nmr spectrum of $^{13}CH_3{}^{13}CO_2H$ enriched coarctatin (**I**). Two strong signals at 88.6 and 166.6 ppm are due to CCl_3CO_2H.

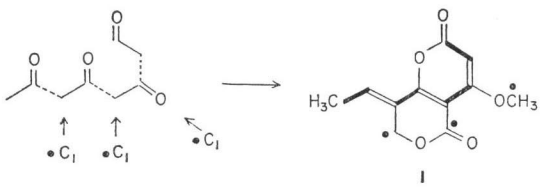


of ^{13}C-^{13}C couplings were observed between quaternary sp^2 carbons. Among these, a peak at 99.2 ppm coupling to C-3 (169.9 ppm) with $J_{C-C}=67$ Hz was unambiguously assigned to C-4. The remaining pair of resonances at 163.5 and 99.2 ppm was therefore due to C-6 and 5. C-10 was assigned to a peak at 162.6 ppm by elimination to give the total ^{13}C assignments of **I**.

^{13}C-Labeled samples of **I** were prepared by separate additions of each *ca.* 90% enriched $CH_3{}^{13}COONa$, $^{13}CH_3COONa$, $^{13}CH_3{}^{13}COONa$ (diluted three fold with unlabeled sodium acetate) and $H^{13}COONa$ at the level of 15 mg/50 ml fermentation broth on 10, 11 and 12 days after inoculation. At the end of the fermentation, ^{13}C-enriched **I** was isolated as reported previously.[2]

In the ^{13}C-nmr spectrum of the $CH_3{}^{13}COONa$ enriched **I**, the signal intensities of C-1, 3, 5 and 7 were increased by 8 times, whereas the resonances due to C-2, 4, 6 and 8 were enriched in the ^{13}C-nmr spectrum of **I** labeled with $^{13}CH_3COONa$. The label of $H^{13}COONa$ was also efficiently incorporated into C-9, C-10, and C-11. Several attempts to label **I** with CD_3CD_2COONa (checked by mass spectrometry), however, were unsuccessful. In the ^{13}C-nmr spectrum of **I** labeled with $^{13}CH_3{}^{13}COONa$, two pairs of ^{13}C-^{13}C couplings, C-1,2 (J=81 Hz), and C-7,8 (J=42 Hz) were observed in addition to those explained previously.

Fig. 2. Biosynthetic pathway of coarctatin (**I**).



Thus, it has been proved that coarctatin is biosynthesized from four acetates and three C_1-units (Fig. 2) in the same manner as most fungal metabolites.

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References

1) For Part X see, SETO, H.; Y. MIYAZAKI, K. FUJITA & N. ŌTAKE: Studies on the ionophorous antibiotics. X. The assignments of ^{13}C-nmr spectrum of salinomycin. Tetrahed. Lett. 1977: 2417~2420, 1977
2) BURROW, B. F.; W. B. TURNER & E. R. H. WALKER: 8-Ethylidene-7,8-dihydro-4-methoxypyrano[4,3-b]pyran-2,5-dione (coarctatin), a

metabolite of *Chaetomium coarctatum*. J. Chem. Soc. Perkin I. 1975: 999~1000, 1975

3) SETO, H. & S. URANO: Revised assignment of the ^{13}C-nmr spectrum of radicinin. Agr. Biol. Chem. 39: 915~916, 1975
TANABE, M.; H. SETO & L. F. JOHNSON: Biosynthetic studies with carbon-13. Carbon-13 nuclear magnetic resonance spectra of radicinin. J. Am. Chem. Soc. 92: 2157~2158, 1970

4) URAMOTO, M.; L. W. CARY & M. TANABE: Abstract of the Annual Meeting of The Agricultural Chemical Society of Japan, p. 153, Yokohama, 1977

5) TORI, K.: Carbon-13 nmr spectral studies of aloenin and its derivatives. Carbon-13 signal assignment problem of 4-methoxy-2-pyrons. Tetrahed. Lett. 1976: 1311~1314, 1976

6) TAKEUCHI, S.; J. UZAWA, H. SETO & H. YONEHARA: New ^{13}C-nmr techniques applied to pentalenolactone structure. Tetrahed. Lett. 1977: 2943~2946, 1977