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ANTITUMOR AGENTS FROM BURSERA FAGAROIDES (BURSERACEAE) (β-PELTATIN-A-METHYLETHER AND 5'-DESMETHOXY-β-PELTATIN-A-METHYLETHER)

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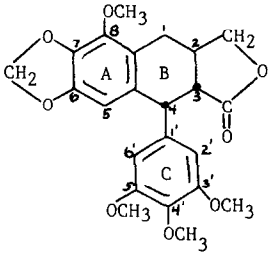
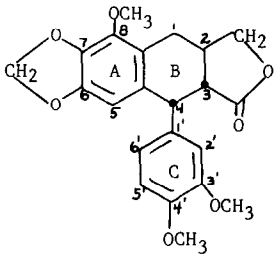
As a result of our continuous screening of Southwestern plants for potential antitumor activity, it was found that the chloroform extract of the Mexican plant, Bursera fagaroides (Burseraceae) demonstrated biological activity in the Walker carcinoma 256 (intramuscular) tumor system (WA16) of the Cancer Chemotherapy National Service Center (CCNSC) at a level of 32% T/C (test/control) at 200 mg./kg. Activity in this system is defined as a percent T/C value of less than 60 in a satisfactory dose response test (1).

Utilizing an elution chromatography system employing an alumina column followed by a Silica Gel G dry column (2) it was possible to separate several single components. Of these, only two compounds demonstrated antitumor activity. These compounds were identified as β-peltatin-A-methylether (I) and a new compound 5'-desmethoxy-β-peltatin-A-methylether (II). Compound I exhibited activity at a level of 10% T/C at 12.5 mg./kg. and II exhibited activity at a level of 20% T/C at 100 mg./kg. in the WA16 tumor system.



COMPOUND I



COMPOUND II

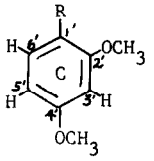
Compound I showed a molecular weight of 428 by means of mass spectrometry. This was compatible with a molecular formula of $C_{23}H_{24}O_8$. An authentic sample of this material was provided by Dr. J.L. Hartwell of the CCNSC. A mixed melting point of I with the authentic sample did not show any depression. The infrared spectra of both compounds were superimposable. The NMR data of I are summarized in the table. Compound I when treated with sodium acetate yielded another compound which was shown to be identical with an authentic sample of β-peltatin-B-methylether. This is the first time that β-peltatin-A-methylether has been isolated from a natural source. This compound, however, has been prepared synthetically (3).

Compound II, which has not been previously reported, was separated as indicated above and was crystallized from methanol and acetone. It had a melting point of 142-143°C which when treated at high vacuum at 100°C for 24 hr. yielded a material having a melting point of 182-182.5°C. Another set of crystals was obtained from the methanol crystallization which melted at 167-167.5°C. These crystals when subjected to high vacuum at 100°C for 24 hr. did not change. All three of the crystals obtained showed different patterns in infrared when KBr pellets were employed. All of them gave a single spot on thin layer chromatography, the same nuclear magnetic resonance spectra, and the same mass spectra. When the three compounds were dissolved in chloroform and subjected to infrared spectrometry, the spectra were identical. The molecular weight of 398 was in agreement with the chemical analysis indicating a molecular formula of $C_{22}H_{22}O_7$.

The analytical data and the nuclear magnetic resonance spectrum of II indicated the presence of three methoxy groups. The signals at 3.76 and 3.80 ppm are assigned to the methoxy groups at C-3' and C-4'; while the signal at 4.1 ppm to the methoxy group at C-8. The presence of the methylenedioxy group in II was revealed by a two proton signal at 5.82 ppm. The aromatic region of the nuclear magnetic resonance spectrum indicated the presence of four protons. A singlet at 6.21 ppm was assigned to the proton C-5. Doublets at 6.66 (ortho-coupling) and 6.92 ppm (meta-coupling) were assigned to the protons C-5' and C-2', respectively. A multiplet of one proton at 6.33 ppm (ortho, meta-coupling) was assigned to the proton C-6'. The table below summarizes the nuclear magnetic resonance data of I and II.

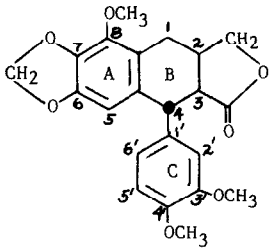
COMPOUND I		COMPOUND II	
Ring A		Ring A	
Methoxy group C-8	4.1 ppm	Methoxy group C-8	4.1 ppm
Methylenedioxy group	5.82 ppm	Methylenedioxy group	5.82 ppm
Proton C-5	6.21 ppm	Proton C-5	6.21 ppm
Ring C		Ring C	
Methoxy groups C-3'	3.70 ppm	Methoxy group C-3'	3.76 ppm
Methoxy group C-4'	3.75 ppm	Methoxy group C-4'	3.80 ppm
Two protons C-2', C-6'	6.31 ppm	Proton C-2'	6.92 ppm (d)
		Proton C-5'	6.66 ppm (d)
		Proton C-6'	6.33 ppm (m)

The fact that the signals for the C-8 methoxy, methylenedioxy, and C-5 proton were exactly the same in I and II proved that ring A in both compounds had the same structure. However, the possibility of meta substitution of the methoxy groups in ring C of II



remained and cannot be conclusively eliminated by the NMR data indicated above. The mass spectrum of II was particularly instructive in eliminating this possibility. The mass spectrum of II showed an intense ion at m/e 138 corresponding to the dimethoxybenzene moiety. Further fragmentation of this moiety established that the dimethoxybenzene is ortho substituted based on the following data. o-Dimethoxybenzene gives fragments of significant abundance at m/e 123 and 95, while m-dimethoxybenzene gives an ion at m/e 95 of significant abundance and one of negligible abundance at m/e 123 (4). In the mass spectrum of II, the abundance of the ions at m/e 123 and 95 is virtually in the same proportion as those recorded for o-dimethoxybenzene. In addition, the mass spectrum of m-dimethoxybenzene shows two abundant ions at m/e 108 and 109 which are insignificant in the spectrum of o-dimethoxybenzene as well as that of II. The fragmentation patterns of I and II were in agreement with the results and interpretation of α and β peltatins as reported by Duffield (5).

The optical rotation of II was $[\alpha]_D^{24°}$ - 146° indicating an A-type isomer (trans 2/3, cis 3/4). After alkali treatment, its optical rotation became positive $[\alpha]_D^{24°}$ + 23° in agreement with its conversion into a B-type isomer (cis 2/3, trans 3/4). Therefore, the following structure was assigned to the B-type isomer of II.



B-TYPE ISOMER OF II

The isomers obtained from the reaction of I and II with sodium acetate did not show antitumor activity.

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