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Inhibition of prostaglandin biosynthesis by derivatives of olivetol formed under pyrolysis of cannabidiol

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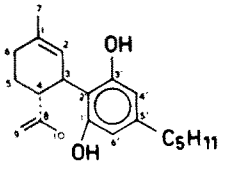
In previous publications [1–3] several components present in *Cannabis sativa* L. have been assayed for their inhibitory activity on prostaglandin (PG) biosynthesis. Burstein *et al.* showed that cannabidiol (CBD, **I**), Δ1(2)-tetrahydro-cannabinol (THC, **II**) and several other cannabinoids are effective inhibitors of PG-synthesis. It was also shown that the inhibitory power of the cannabinoids resides in the phenolic moiety of the molecule; thus olivetol (**III**) showed a higher potency than the cannabinoids [2].

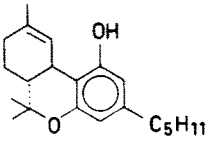
Since cannabis is generally smoked we prefered to study the components obtained by pyrolytic treatment of cannabinoids for their PG-inhibitory activity. We have shown that after pyrolysis of cannabidiol several new cannabinoidal components and phenolic cracking products are formed. The structures of the majority of these components have been elucidated and described in preceding articles [4–8]. Until now the following new compounds have been identified in the nitrogen and air pyrolysates of CBD: olivetol, 2-methylolivetol (**IV**), 2-ethylolivetol (**V**), 2,2-dimethyl-5-hydroxy-7-pentylchromene (**VI**), 4-hydroxy-6-pentylbenzofuran (**VII**), 1,2-(or 1,4)-benzopyran (**VIII**), Δ4(5)CBD, 3,4-*cis*-Δ1(6)CBD, a bicyclic cannabinoid **IX**, and two unusual rearrangement products **X** and **XI**. In view of the structural resemblance of all these products with olivetol it was of interest to study their effects on PG-synthesis. For this purpose the products **III**–**VII** and **XI** were synthesized, while compound **X** was still available from a previous isolation. The synthesis of these products will be described elsewhere.

Procedure [9]. Prostaglandin biosynthesis was assayed by continuous measurement of O_2-consumption by polarography in an Oxygraph (Gilson Medical Electronics, Middleton, WI) equipped with a Clark electrode, cell volume 2.0 ml. Lyophylized microsomes from sheep seminal vesicles (1 mg protein) were incubated at 26° in 1.9 ml 0.2 M Tris–HCL buffer (pH 8) with 200 μg glutathione, 50 μg hydroquinone and varying amounts of inhibitor for 90 sec. The reaction was started by the addition of 40 μg 8,11,14-eicosatrienoic acid dissolved in 0.1 ml buffer solution. The velocity of the O_2-uptake was plotted against the logarithm of inhibitor-concentration. From these graphs the values of concentrations giving 50 per cent inhibition (ID_{50}) were calculated.

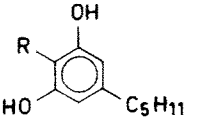
Results and discussion. In addition to the products mentioned above (**III**–**VII**, **X**, **XI**), indomethacin [10], a potent inhibitor, CBD, Δ1(2)THC, Δ1(6)THC, cannabinol (CBN),



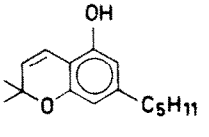
I



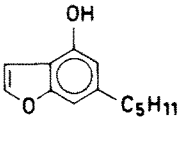
II



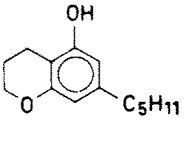
III R = H
IV R = CH_3
V R = CH_2CH_3



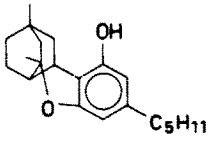
VI



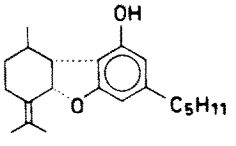
VII



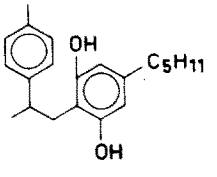
VIII



IX



X



XI

Table 1.

	ID 50
Indomethacin	1×10^{-6} M
2-Methylolivetol (IV)	5×10^{-6} M
2-Ethylolivetol (V)	8×10^{-6} M
Olivetol (III)	13×10^{-6} M
4-Hydroxy-6-pentyl-benzofuran (VII)	15×10^{-6} M
Compound XI	24×10^{-6} M
Cannabidiol (I)	60×10^{-6} M
Cannabinol	60×10^{-6} M
Δ1(6)Tetrahydrocannabinol	70×10^{-6} M
Resorcinol	70×10^{-6} M
Pyrocatechol	70×10^{-6} M
2,2-Dimethyl-5-hydroxy-7-pentylchromene(VI)	80×10^{-6} M
Compound X	110×10^{-6} M
Δ1(2)Tetrahydrocannabinol (II)	110×10^{-6} M
Hydroquinone	360×10^{-6} M

pyrocatechol and resorcinol were also tested for comparative reasons. The results of these measurements are given in Table 1. The ID_{50} values found for the known cannabinoids, CBD, Δ1(2) and Δ1(6)THC, CBN, olivetol and indomethacin compare well with those found by Burstein [2] although he derived the synthetase from bull seminal vesicles. It can also be seen that there is a drastic increase in potency going from the cannabinoids to the more simple olivetol-derivatives.

The higher activities found for 2-methyl, 2-ethylolivetol and olivetol relative to the activities found for Δ1(2)THC, Δ1(6)THC, CBN and the products VI and X suggest that the inhibiting-power is most pronounced if there are two free hydroxyl groups.

In conclusion, the results presented above show that the phenolic cracking products formed by pyrolytic treatment of CBD are strong inhibitors of PG-biosynthesis under the conditions described. The data presented here may lead to a better understanding of the effects observed with cannabis smoke.

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Effect of some mucopolysaccharides on activated factor X

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A method for purifying the activated factor X (Xa) from bovine thrombin, was developed by Yin and Wessler in 1968.

The procedure [1] utilized a DEAE-cellulose column, from which three eluates were obtained, with 0.1 M NaCl (pH 7), 0.14 M NaCl in 0.05 M sodium citrate (pH 5.8), and with sodium citrate pH 5.8 respectively. The three eluates were tested for clotting activity; in the first fraction, only thrombin was present; in the second thrombin and factors II, VII IX and non-activated factor X were detected; the third eluate contained only activated factor X.

The Xa obtained with this procedure failed to induce clotting of fibrinogen standard preparations after a 24 hr incubation at 22°, or at 37°, with or without calcium, even when Na citrate was removed by dialysis against NaCl 0.14 M.

Yin, Wessler and Stoll later developed another procedure for extracting an Xa inhibitor from rabbit plasma and evaluated its biochemical properties [2–4].

As a result of several physical–chemical and biological tests, they concluded that the biological activities defined as either inhibition of Xa, or antithrombin III, or heparin cofactor activity, are due to a single inhibitor present in plasma that is able to block the activity of factor Xa, as well as that of thrombin. The inhibition is 30 times as active against Xa as against thrombin. When optimum amounts of heparin and Xa inhibitor are present, the inhibition of Xa is progressive and irreversible. The Xa clotting activity is not restored even by addition of protamine sulphate which is known to inhibit heparin. When heparin and Xa inhibitor are present, the inhibition of thrombin is progressive and reversible; in fact the clotting activity