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

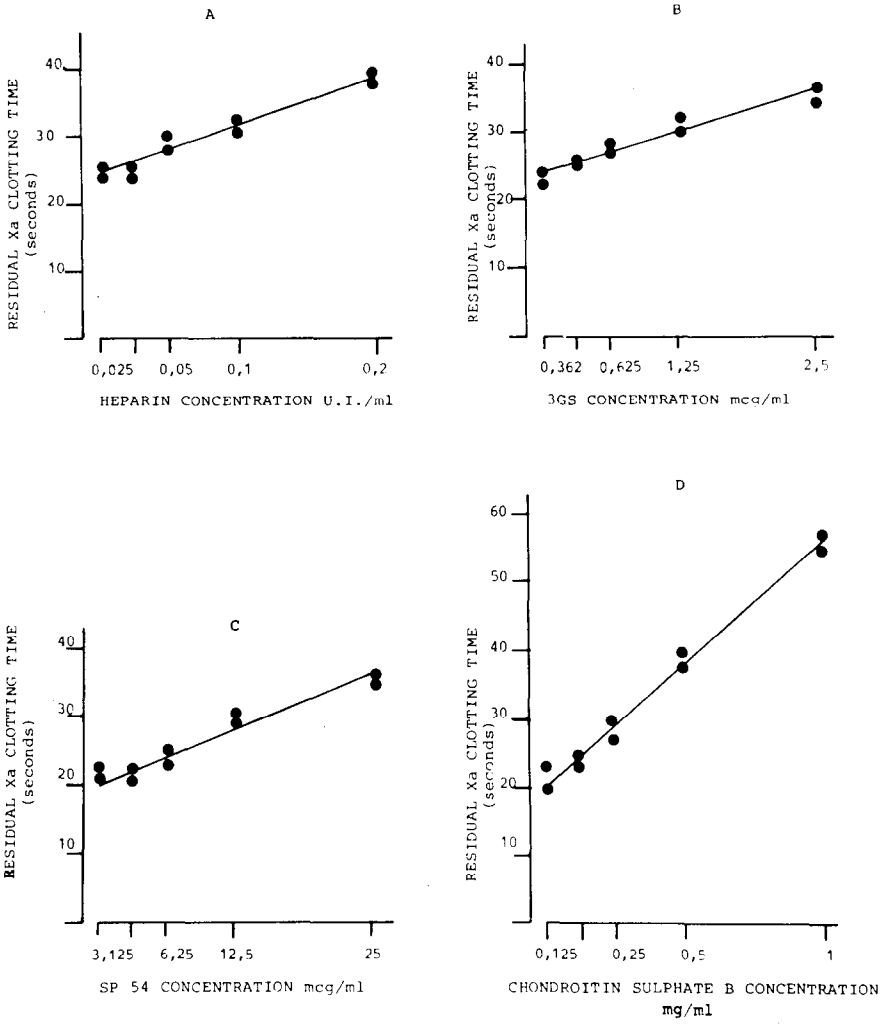

Fig. 1. (a) Dose–response curve of *heparin*; (b) dose–response curve of *3GS*; (c) dose–response curve of *SP 54*; (d) dose–response curve of *chondroitin sulphate B*.

of thrombin can be restored by neutralizing heparin with protamine sulphate.

Yin and Wessler showed that a correlation exists between the inhibition of Xa and the presence of heparin and developed a method for assaying heparin plasma [5–6].

In our investigation we have also tried to extend this method for the heparin-like mucopolysaccharides (heparinoids). Among them, the most representative ones, glucuronylglycosaminoglycan sulphate (3GS), pentosane polisulphoester SP 54 (mol wt 2000) and A, B and C chondroitin sulphate have been selected.

Injectable heparin (Liquemin 5000 i.u./ml) (Roche). Glucuronylglycosaminoglycan sulphate (3GS) purified from a commercial preparation (Vessel-Alfa Farmaceutici Bologna) by chromatography on microcristalline cellulosa (Merk-Co. Darmstadt) column (our preparation n. 24-1). Pentosan polisulphoester SP-54 mol. wt 2000, injectable Hemoclar (Laboratories Clin-Comar-Byle-Paris). Chondroitin sulphate A (Research Division Miles Laboratories Ltd. batch WP 4307). Chondroitin sulphate B lot S 3101 and C lot S 3502 (Sekagaku Kogyo Co. Ltd. Tokyo). Tris maleate buffer (mono Tris (hydroxymethyl) aminomethane) 0.02 M pH 7.5 and $CaCl_2$ reagent grade (0.025 M in deionized water) were employed during the clotting test. Both reagents were kept at 37° during the experiments. Plasma Cephalin Reagent (PCR) containing cephalin extracted from rabbit brain dissolved in heparin free bovine plasma (Sigma Chemical Co. St Louis). During the experiments PCR was kept at 37°. Activated Factor X (Xa) 4 Units of Factor Xa and 10 mg of bovine serum albumin were contained in 1 ml of Tris maleate buffer (Sigma Chem. Co.). This solution was kept at 4° during the experiments. Normal Human Plasma (NHP). Blood samples were withdrawn from a minimum of 6 healthy volunteers who had not taken any drug for at least a week. Blood samples were diluted 1:10 with Na citrate 3.8%. After centrifugation plasma samples were pooled and used on the same day.

Dose-response curves of heparin. Heparin was diluted in NHP to obtain 0.2, 0.1, 0.05, 0.025 i.u./ml. Aliquots of 0.5 ml of the solution thus obtained were placed in a test tube kept at 37° in a water batch and 0.1 ml NHP and 0.3 ml of Tris maleate buffer were added. The mixture was shaken for 1 min then 0.1 ml of Xa solution was added and after thorough mixing, let stand for 90 sec. At the end of this period 0.1 ml was transferred into a test tube (previously warmed to 37°). 0.1 ml of $CaCl_2$ (0.025 M) was added and after 10 sec 0.2 ml of PCR. From this point on, the clotting time was recorded and then plotted against the heparin dose. Dose–response curves for heparinoids were determined by the same procedure, except for dilutions which were suitably chosen in order to give clotting times within the range considered for heparin.

Figure 1a shows the inhibition of Xa factor by heparin.

For every dose two groups of three determinations were made on two subsequent days. It can be seen that the dose–response relationship is very close to linearity ($v = 0.965$; P 0.001). Also the agreement between our results and those of Yin *et al.* [6] is remarkable. The most recent views on the effect of heparin-like substances on Xa recognize the inhibition of the latter by the former. Our results confirm these views: a clear-cut inhibition of Xa by 3GS at various doses is obtained *in vitro*, and the dose–response relationship is linear (Fig. 1b).

The comparison of the slopes of the curves of heparin and 3GS enables us to express the concentration of 3GS in terms of i.u./ml. This is of course somehow arbitrary because these i.u./ml are defined on the basis of the mutton plasma coagulation test and they are applied to 3GS on the basis of an Xa inhibition test. The remarkable fact is that the titre of our sample obtained in this way (80 i.u./mg) is in very good agreement with that obtained by the currently used titration test of USP.

From our viewpoint these findings provide positive evidence that Xa plays a basic role in the process of coagulation, and it may well be possible that the effect of heparin like anticoagulants, such as 3GS, as measured by the mutton plasma coagulation test are due to the inhibition of Xa factor as well as to the inhibiton of thrombin.

The same considerations apply to SP 54. In fact, the same procedure as used for 3GS applied to its dose–response curves (Fig. 1c) leads to the definition of an i.u., which in turn yields a titre of the sample (8 i.u./mg) in agreement with that declared by the manufacturer. Chondroitin sulphate A and C showed no detectable inhibitory activity. On the contrary some slight inactivation of Xa by chondroitin sulphate B at relatively high doses was detected (Fig. 1d). The titre of the sample, in terms of anticoagulant activity, was 0.4 i.u./mg. This finding could of course be due to contamination by other heparin-like mucopolysaccharides, as already reported by some authors [8].

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