The Biological Effects of the Bark of *Saccoglottis Gabonensis* Urban (Humiriaceae) on Microorganisms

The use of plants and herbs in the treatment of disease is widespread in the oriental contries. In Nigeria, extracts from leaves and barks of trees such as *Alstonia boonei, Khaya ivorensis*, and *Khaya senegalis* etc. and various types of roots are used for the treatment of malaria fever.

Saccoglottis gabonensis Urban belongs to the family Humiriacea. The bark is reddish-brown exuding amber coloured sap. It imparts a bitter taste to plam wine, a popular drink among Nigerian peasant farmers.

This paper describes the effects of fresh extracts from the bark on the bacterial population in palm-wine, and on the intestinal microflora of the consumer.

Preparation of bark extract. 60 g of the ground bark of *Saccoglottis gabonensis* was extracted with about 200 ml of solvent. Each extract was concentrated in vacuo to 30 ml. The solvents used were light petrol (40°–60 °C), ethanol, ethyl acetate and methanol.

Isolation of microorganisms. The bacteria (*Leuconostoc mesenteroides* and *Lactobacillus plantarum*) were isolated from palm-wine by culturing them on ROGOSA, MITCHELL and WISEMAN medium[1]. *Streptococcus faecalis* was obtained from a patient in the teaching hospital. The organism was grown on Hannay and Norton's sodium azide medium (pH 7.5) as described by BAKER[2].

Test for inhibitory action of extracts. Cells from 18-hour cultures of test organisms (*Leuconostoc mesenteroides, Lactobacillus plantarum* and *Streptococcus faecalis*) were washed, resuspended in sterile saline and the concentration adjusted to 10^4/ml.

19 ml agar was inoculated with 1 ml of the suspension of the bacteria cells in saline (10^4/ml) and was placed in sterile petri dishes. Sterilized paper discs (10 mm diam.) which have been soaked in the bark extracts and dried in hot petri dishes, were placed directly on the surface of the agar. Sterilized paper discs in the solvent blanks were similarly treated. The plates (both tests and blanks) were incubated at 37 °C for 2 days and the inhibition zones measured accurately with a planimeter.

Identification of active compounds in extracts. When a concentrate of methanol extract was left at room temperature (22–25 °C) for about 2 weeks, big snow-white crystals separated. On recrystalling from ethanol it came down as white crystalline powder. The compound was found to correspond to spot III on the chromatogram (Table II). The Rf value was 0.45 in isopropanol-formic acid-water (2:5:5 v/v). Results of various physicochemical tests (IR, UV, NMR, action of alkali to give bathochromic shift of UV-peak etc.) indicated that it was bergenin. The compound was reported to be present in the plant's bark by OGAN[3].

Spot I. (Rf 0.20) was greenish-yellow. The IR-functional peaks and the UV- absorption peaks of this compound at 237, 280, 330 and 400 nm point to the fact that is a substituted α-naphthoquinone.

Spot II (Rf 0.30) was dark brown on the chromatogram. The compound was obtained as a white amorphous powder when purified by recrystallisation from methanol and ethanol. The compound was identified as *ethyl ester* of *gallic acid.*

Compounds on spots IV and V are in the supernatant of methanol, ethanol or ethyl-acetate extracts after the separation of other solids. They gave blue fluorescence under the UV-light. When the chromatogram was briefly immersed into ammonium fume spot V turned to green fluorescence. No analytical test has so far been performed, but the change to green fluorescence is characteristic of some phenolic acids.

Metabolic studies. Male rats of approximately 200 g was anaesthetized by i.p. injection of 25% urethane solution (6 ml/kg). Bile samples were collected from biliary canulated rats according to the procedures of BOYLAND, RAMSAY and SIMS[4].

10 mg of a fluorescent extract spot IV described above, was obtained from the bark of *Saccoglottis gabonensis* Urban (Humiriaceae) and injected i.v. into the experimental rat; bile samples were collected at intervals for 3 h.

Paper chromatography of these bile samples showed that the fluorescent materials were rapidly excreted in the bile within the 1st h, and only traces were detected in the 2nd and 3rd. The excretory product was fluorescent, but gave a lower Rf value (0.56) compared with the original product (Table II). This suggests that the compound has been converted to a metabolite. The pathway for the metabolism of this compound is being investigated.

In the adult human, the large intestine has an aboundant bacterial flora, while the numbers in lower ileum are much smaller and the upper small intestine is practically sterile. Several different species are represented, particularly coliform bacilli of different types, Enterococci, Staphylococci of the aureus and albus varieties, anaerobic spore-bearing organisms such as *Cl. velchii* and *Cl. putrifaciens*, aciduric bacteria including *L. acidophillus* and *L. brevis*, spirochaetes and yeast.

The effect of the plant extracts on *Streptococcus feacalis* showed a similar inhibitory reaction, as in the case of the two bacterial species of palm-wine (Table I). However, excretory product of the fluorescent compound IV failed to inhibit the intestinal bacterial species. Thus it is possible that these other plant constituents might have been metabolized to inactive compounds.

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Table I. Effect of extracts on zones of inhibition

Organisms	Diameter of zones of inhibition (cm)			
	MeOH extract	Ethyl acetate Extract	Ether Extract	Ethanol Extract
Lactobacillus plantarum	6.5 ± 0.2	3.5 ± 0.1	0	4.3 ± 0.2
Leuconostoc mesenteroides	4.8 ± 0.3	3.0 ± 0.2	0	3.4 ± 0.3
Streptococcus faecalis	2.9 ± 0.2	1.8 ± 0.3	0	2.1 ± 0.3

Table II. Effect of active principles in extracts

Organisms	Ability to inhibit growth of bacteria					
	I	II	III	IV	V	B[a]
Rf[b]	0.20	0.30	0.45	0.62	0.85	0.56
Lactobacillus plantarum	+	+	−	+	−	
Leuconostoc mesenteriodes	+	+	−	+	−	
Streptococcus faecalis	+	+	−	+	−	−

[a] B is the fluorescent biliary excetory product. [b] Rf in isopropanol-formic acid-water (2:5:5 v/v).

The effects of the plant extracts on other bacterial species must be studied in vitro and in vivo to have an idea of the possible effect of the bark on human gut flora.

Résumé. Les extraits de l'écorce de *Saccoglotis gabonensis* Urban empêchent l'accroissement de la bactérie du vin de palme (une boisson alcoolique) et du *Streptococcus faecalis* isolé de l'intestin. Les effets métaboliques des extraits de composés fluorescents de cette écorce sur les flores gastro-intestinales sont discutés.

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PRO LABORATORIO

The Use of Diazepan as Premedication in Pentobarbital Anaesthesia in Guinea-Pigs

It is known how difficult it is to obtain a good anaesthetic plane in the guinea-pig, necessary to perform short or long surgical prodecures, when pentobarbital, chloralose or urethane are used[1–10].

With pentobarbital the results are unpredictable; high doses are usually lethal, due to marked depression of the respiratory centers; on the other hand, low doses might be inadequate to produce deep anaesthesia; supplementary doses, administered intraperitoneally are not under control; in some cases even after a 2nd or 3rd dose, the animal is not in the surgical plane, whereas in others, 1 supplementary dose may kill the animal.

The use of adjuvants has permitted to reduce the dose of the most commonly used anaesthetics. Maykut[1] has combined pethidine (meperidine) and pentobarbital; the potentiation produced made it possible to reduce the dose of pentobarbital. Chloral hydrate has also been used with pentobarbital; however, supplementary doses of chloral hydrate were usually needed when anaesthesia was not deep enough after 20 min[2]. Dolowy and Hesse[3] have used chlorpromazine as premedication in doses of 25 mg/kg followed by pentobarbital 30 mg/kg. Other authors[4] have associated pentobarbital and ethyl alcohol without achieving the desired potentiation.

The effect of diazepan as premedication followed by anaesthesia with pentobarbital was investigated in guinea-pigs.

Materials and methods. Guinea-pigs of either sex, weighing 250–900 g were used. The control group received only pentobarbital i.p., and the study group was firstly

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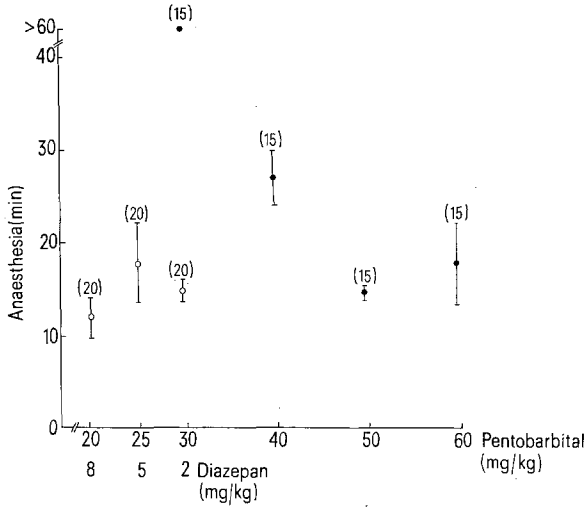

Fig. 1. Beginning of the surgical anaesthetic plane in guinea-pigs. Full circles, pentobarbital i.p.; open circles, diazepan i.m. followed 30 min later with pentobarbital i.p. In brackets, number of animals; vertical bars, standard deviation.

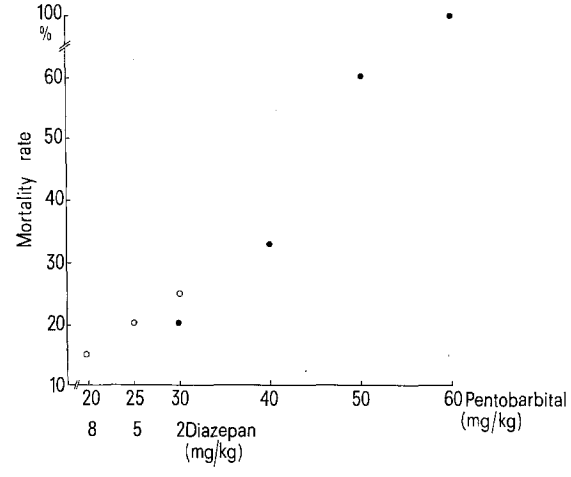

Fig. 2. Mortality rate (in percentage) of guinea-pigs. During and after the 1st h. Full circles, pentobarbital i.p.; open circles, diazepan i.m. followed 30 min later by pentobarbital i.p.