2.6, 2 h). In heart and liver, increased 5-HT-^{14}C levels were still observed after perfusion (Table I).

Eight h after administering tritiated DMCI, the radioactivity in rat heart was due essentially to the unchanged compound. Thus, the inhibition of rat heart MAO was most likely produced by DMCI and not by a metabolite. In the bile collected from cannulated rats, a total of 32% of administered radioactivity was recovered throughout 8 h (Table II). The major metabolite in bile was identified as the glucuronide of 2-methyl-9-hydroxymethyl-β-carbolinium iodide, a product resulting from the oxidation of the 9-methyl group of DMCI. About 30% of the administered radioactivity was excreted in the urine of cannulated rats during the 8-h period; in the urine the glucuronide of the 9-hydroxymethyl metabolite accounted for 13% and the unchanged DMCI, 87% of the radioactivity.

In view of the results of this study, DMCI exhibits properties required of a peripheral MAO inhibitor. It is significant that this compound demonstrates considerable inhibition of heart MAO which is thought to be representative of the enzyme located in the peripheral nervous system.

Resumen. Yoduro de 2,9-dimetilo-carbolinio (DMCI) y no sus metabolitos inhibió la enzyma monoamino oxidasa y elevó ^{14}C-serotonia en el corazón e higado, pero no en el cerebro de ratas.

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The Effect of the Carbohydrate Moiety on Biological Activities of Synthetic Glycosides of Medicagenic Acid

The various biological activities exerted by saponins are generally attributed to their aglycone moieties, whereas to the composition and constitution of their carbohydrate moieties only small, if any, significance is assigned[1,2].

With regard to lucerne saponins, it was found[3,4] that their fungistatic and haemolytic activities are dependent solely on the presence of one aglycone, medicagenic acid, which is a dicarboxylic triterpene acid. The structure of a highly active lucerne saponin was recently established as 0-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosylmedicagenic acid[5]. In continuation of this work, the synthesis of medicagenic acid containing glycosides, the carbohydrate moieties of which are composed of 1 or 2 sugars, either α- or β-linked, was undertaken, and the influence of the sugar moiety on the extent of the fungistatic and haemolytic activities was examined. Medicagenic acid was prepared as described[3] and its carboxyl groups converted to the benzohydryl ester[6]. The following sugars were used: glucose, galactose, maltose, cellobiose and gentiobiose. They were converted to their acetobromo derivatives as described by JEANLOZ and STOFFYN[7]. The synthesis of the glycosides was carried out by the modified Koenigs-Knorr method[8], as described also by MORRIS and TANKERSLEY[6] for the synthesis of a β-D-glucoside of medicagenic acid. After removal of the benzohydryl groups by catalytic hydrogenolysis, the synthetic saponins were purified by thin layer chromatography on Kieselgel HR plates, using the mixture of ethylacetate-acetic acid-water (7:2:2) as solvent. The saponins containing only 1 sugar residue had an Rf value of 0.85, and those which contained one of the disaccharides had Rf values of 0.80–

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Haemolytic and fungistatic activities of native and synthetic glycosides and of medicagenic acid assayed with ram red blood cells[a] and *Sclerotium rolfsii* Sacc.[a]

Compound assayed	Mode of linkage in carbohydrate moiety	Haemolytic index	Mycelial growth inhibition by $1 \times 10^{-4}M$ saponin in the culture medium (% of control)
Medicagenic acid	—	6600	92.5
Native lucerne saponin	β 1→6, β 1→3	15000	88.0
Glucose-medicagenic acid	—	13500	83.7
Galactose-medicagenic acid	—	14000	88.3
Maltose-medicagenic acid	α 1→4	14600	88.6
Cellobiose-medicagenic acid	β 1→4	14200	86.2
Gentiobiose-medicagenic acid	β 1→6	14000	86.5

[a] For methods, see GESTETNER et al.[3].

0.85. Samples of the synthetic glycosides thus prepared were subjected to acid and enzymatic hydrolysis, quantitative determination of the sugar residues with 3:5 dinitrosalicylic acid[9] and of medicagenic acid[10]. A 1:3 (w/w) ratio of glucose to medicagenic acid was found in the acid hydrolysate of the saponins which has incorporated monosugars, whereas a 1:1.5 (w/w) ratio was found for those which had employed disugars, indicating the attachement to medicagenic acid of either a monosugar or a disugar, as expected. Yeast α-glucosidase was ineffective in attempts to cleave the glucosidic bond between sugar and aglycone. On the other hand, incubation with almond β-glucosidase resulted in the liberation of medicagenic acid from the synthetic saponins. During the synthesis of the saponins, no attempt was made to block the axial 2β-hydroxy group of medicagenic acid, since steric interference would make substitution at this position virtually impossible.

The haemolytic and fungistatic activities of the synthetic glycosides as compared to the native lucerne saponin and medicagenic acid were assayed with ram red blood cells and with the fungus *Sclerotium rolfsii* Sacc. As shown in the Table, the fungistatic activities of the synthetic saponins are similar to each other and in the same order of magnitude as that of the native lucerne saponin. The fungistatic activity of the glycosides is less than that of the aglycone-medicagenic acid itself. As for haemolytic activity, in this instance medicagenic acid is less haemolytic than its glycosides, but the activities of all the glycosides, the native as well as the synthetic ones, are very similar to each other.

Thus it seems safe to conclude that as long as the carbohydrate moiety of the synthetic glycosides is composed of 1 or 2 hexoses, and does not introduce extreme changes in some properties, e.g., solubility of the glycoside in biological media, it will have no considerable effect on its biological activites. A reasonable interpretation of the results given in the Table would be that with regard to fungistatic activity the extent of solubility of medicagenic acid in the culture medium is optimal for exerting its growth-inhibiting activity. By converting it to a glycoside, its lypophylic nature is changed to a more hydrophylic one, thus interfering with its access to the lipid constituents of the cell membrane and consequently the glycosides will have a lesser activity than the aglycone. On the other hand, the haemolytic activity of saponins is measured on washed red blood cells in isotomic buffer solutions, i.e., not in their natural medium. In this case the solubility of the glycoside in the buffer seems to be of a greater importance than its interacting ability with membrane constituents. As the solubility of medicagenic acid in this medium is considerably smaller then its corresponding glycoside, its activity will be less than that of the glycosides.

Zusammenfassung. Synthetische Glycoside der Medicagensäure wirken fungistatisch und hämolytisch im gleichen Ausmass wie das natürliche Luzerne-Saponin und unbeeinflusst von der Natur des Zuckers in den Glycosiden. Der hämolytische Index der Medicagensäure ist geringer, die fungistatische Wirkung derselben aber etwas höher als diejenige der Glycoside, was mit der geringeren Wasserlöslichkeit der Medicagensäure erklärt werden kann.

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5-Methyl-3-Butyl-Octahydroindolizine, a Novel Type of Pheromone Attractive to Pharaoh's Ants (*Monomorium pharaonis* (L.)

The tropical Pharaoh's ant, *Monomorium pharaonis* L., is a pest in heated buildings, bakeries, etc, in many non-tropical countries. Especially in hospitals it is a threat to public health. A recent investigation[1] in 9 British hospitals of these insects' long-standing infestations showed that they carry pathogenic bacteria, have the capacity to transmit disease and are able to enter even highly sophisticated isolation units. The small worker ants can find their way through minute holes, even through bandages. Their nests, containing the queens, are mostly hidden at places where they can hardly be found. The usual means to control ants in houses fail when applied to Pharaoh's ants, especially in long-standing infestations. It is therefore important to detect any infestation at an early stage.

This prompted us to look for new ways to detect and control Pharaoh's ants by applying the chemical signals they use for transmitting information (semiochemicals[2]) particularly those used for communication within a species (pheromones).

Pharaoh's ants are known to produce a relatively persistent odour trail[3]. Our preliminary experiments showed that the ants follow a trail made from a hexane extract of paper strips taken from their rearing-boxes. Only in 1 ant species, *Atta texana*, has a trail pheromone been identified: methyl-4-methylpyrrole-2-carboxylate[4].

The activity of the paper extract could also be demonstrated by a choice test, similar to the bioassay, described below. After 75 workers had been released in the dish (Figure), within 15 min 55 of them assembled in the tube containing a paper strip impregnated with the extract, and only 15 in the tube containing blank paper.

In order to isolate the relevant pheromone(s) directly from the insects, they were mass-reared as described by BUSCHINGER[5]. About 6,000 worker ants (1 g) were homogenized and extracted with about 10 ml of CH_2Cl_2. The extract was subjected to gaschromatography (GLC) on 5% OV-17 at 120 °C and fractions of the effluent were collected in capillary tubes. To determine specific biological activities, and monitor further isolation and purification procedures, the bioassay shown in the Figure was used.

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