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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In this choice test, the sample is applied evenly over the surface of a paper strip of 10×0.4 cm, which is then introduced into 1 of 2 tubes connected in a V-shape to a dish. The other tube contains a blank strip. In the dish, 25 worker ants are released and, during 15 min in each of the tubes, the ants are counted every min. The differences between the average numbers present in each of the tubes are used as a measure of activity.

This activity was 5.8 for 10 µl of the crude extract of the insects. After injection on the OV-17 column, highest activities (8.0, 7.4 and 8.6) were found in fractions 1, 4 and 7, corresponding with retention times of 6.4–8.4, 14.1–18.4, and 33.2–48.6 min, respectively. As the first fraction showed only 1 peak, representing about 0.25 µg, the isolation and identification of this compound was attempted first. This was done by further purification of combined similar fractions by GLC on 5% DEGS and 5% OV-101 columns, and determination of MS, IR- and NMR-spectrum.

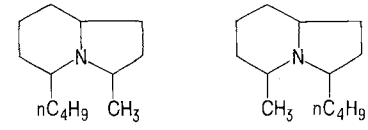
The purified compound showed an activity of 10.2 for 0.25 µg and 13.5, for 3.0 µg. Its Kovats Retention Indices were 1596, 1380 and 1345 on the DEGS, OV-17, and OV-101 columns, respectively.

The mass spectrum, determined by combined GC/MS using several types of apparatus, showed a highest *m/e* value of 195. Moreover, peak matching revealed that this corresponded with an elementary formula of $C_{13}H_{25}N$. Assuming this to be the parent peak, the compound had to be a saturated bicyclic one, as the other spectral data and hydrogenation experiments showed absence of double bonds. The nitrogen is bound to be present in a tertiary amine, because the IR-spectrum showed no bands typical for NH- or NH_2-groups.

A peak found at $m/e = 194$ (M–H), which is higher than the parent peak in the mass spectrum, is characteristic of a nitrogen-containing ring. Prominent peaks were found at $m/e = 180$ ($M\text{-}CH_3$) and $m/e = 138$ (base peak; $M\text{-}C_4H_9$, confirmed by a metastable transition). This is indicative of a methyl and a butyl group attached to the α-C atoms, as fragmentation of amines in the mass spectrometer is dominated by fission of α–C–C bonds.

Indeed the NMR-spectrum (solvent $CDCl_3$) showed 2 methyl groups (triplet at δ 0.88, $C\underline{H}_3\text{–}CH_2$; doublet at δ 1.18, $C\underline{H}_3\text{–}CH\text{–}N$) and moreover 3 $\overline{CH}$ groups adjacent to N (broad 1 proton signals at δ 2.13; δ 2.27, $C\underline{H}\text{–}CH_3$, concluded from a spin-decoupling experiment; δ 2.52).

All these data together are most consistent with indolizine derivatives (I) and (II), which both are unknown in the chemical literature.



(I) (II)

To synthesize (II), 4-aminooctanal diethylacetal was condensed with diethyl-3-oxo-glutarate and ethanal to yield 5-methyl-3-butyl-6,8-dicarbethoxy-7-oxo-octahydroindolizine, which was subsequently saponified, decarboxylated and reduced to (II). On GLC it appeared to contain a number of componds among which several isomers of the pheromone (demonstrated by GC/MS). One of them showed identical retention time, MS and IR-spectra as the isolated pheromone and was biologically active. Analytical details will be published elsewhere[6].

This is probably the first time that a pheromone with an indolizine skeleton has been found. Two components present in other GLC fractions from the ant's extract have also been isolated, and their structures partially elucidated. Their elementary formulae are $C_{13}H_{27}N$ and $C_{15}H_{29}N$. Further details on these compounds, on the stereochemistry of (II) and on biological aspects will be published elsewhere.

Zusammenfassung. Aus Arbeiterinnen der Ameise *Monomorium pharaonis* (L.) wurde 5-Methyl-3-butyl-octahydroindolizin isoliert. Dieses Pheromon übt eine starke Lockstoffwirkung auf die Arbeiterinnen dieser Ameisenart aus. Die Struktur wurde durch Spektralanalyse und Synthese aufgeklärt. Unseres Wissens ist dies das erste Pheromon aus Insekten mit dem Indolizingerüst.

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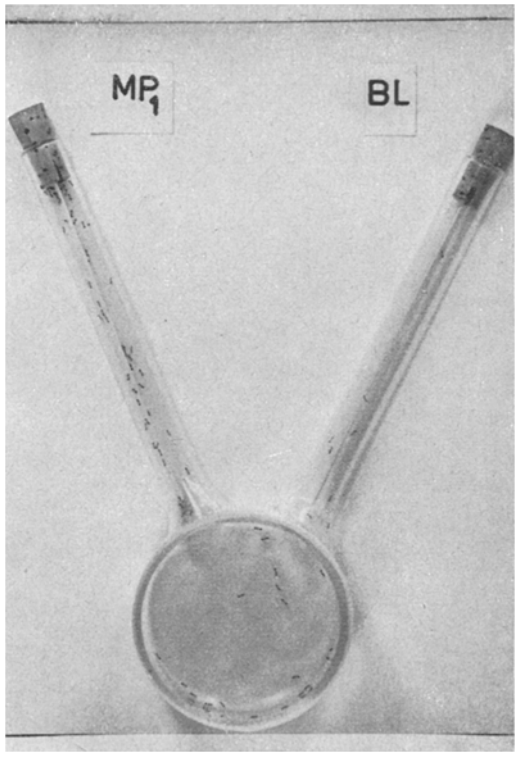

Choice test used for determining the biological activity $MP_1 = 5$ µg pheromone $C_{13}H_{25}N$ (I). BL = blank. Delft, 21-12-1972 Ri/AP.

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