(±)-Des-N-Morphinan: a Unique Bridged Hydrocarbon Attractant for the Rhinoceros Beetle, *Oryctes rhinoceros*;[1] and Development of an Olfactometer[2]

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ABSTRACT

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An olfactometer designed for *Oryctes rhinoceros* (L.) is described and its effectiveness demonstrated by a comparison of results with field tested chemicals. Using the olfactometer, (±)-Des-N-morphinan, but not its stereoisomer was found to be a strong attractant for adult rhinoceros beetles. This is the 1st synthetic bridged hydrocarbon found to be an insect attractant.

A search for possible chemical attractants for the economically important coconut rhinoceros beetle, *Oryctes rhinoceros* (L.), was first reported by Cumber (1956), who tried several olfactometer designs with no apparent success. Work in this area was discontinued until 1966 when a joint United Nations-South Pacific Commission Project was initiated for control of the beetle (Catley 1966). This group evolved a method for field testing potential chemical attractants, which led to the discovery of several effective compounds, most notably derivatives of chrysanthemic acid (Barber et al. 1971, Maddison et al. 1973). Although the field bioassay method most nearly simulates natural conditions, it requires the use of large amounts of test material, which inhibits investigation of possible natural attractants, or chemicals not readily available. Reported here is the development of an olfactometer that can be used to test the response of *O. rhinoceros* to small amounts of chemicals, or extracts of natural material.

In our search for chemical attractants to aid in controlling this insect, we have discovered (±)-des-N-morphinan [(±)-4b,5,6,7,8,8aβ,9,10-octahydro-9β,4b β-propanophenanthrene] (I) to be an excellent attractant in laboratory trails using the olfactometer described. Compound I had been previously observed to be attractive to other insects, tentatively identified as members of the family *Dolichopodidae*, Thyrpticus (Cherian 1974). To our knowledge, this is the 1st synthetic bridged hydrocarbon attractant yet reported. We have also determined that the rhinoceros beetle is able to differentiate between the 2 stereoisomers, (I) and (±)-des-N-isomorphinan [(±)-4b,5,6,7,8,-8aα,9, 10-octahydro-9β, 4bβ-propanophenanthrene] (II).

Materials and Method

The rhinoceros beetle adults used in conjunction with the bioassay were obtained by rearing field collected larvae and pupae in the laboratory, or from the UNDP/FAO project for research on the Control of the Coconut Palm Rhinoceros Beetle, Apia, Western Samoa. The adult beetles were maintained in groups of 25–35, with equal numbers of males and females, which were maintained at suitable levels by adding newly emerged adults as older members of the group died. Consequently, each cage contained individuals of varied age.

At the bottom of each cage was a countersunk plastic bin containing moist, partially decayed sawdust in which the beetles spent most of their time. They were fed on sugarcane and bananas, and had the opportunity for flight exercise. The groups functioned normally with a lifespan of 3–6 mo, similar to that reported by Hurpin and Fresneau (1967).

Fig. 1 shows the "Y" olfactometer designed for the continuous introduction of individual beetles. Glass was used throughout, bonded with a silicon rubber adhesive. The outer sides and the bottoms of the 3 runways were covered with black tape to avoid possible phototaxis (Goonewardene 1959, Goonewardene and Kirthisinghe 1960, Goonewardene and Abeywardena 1960, Maddison 1972); walking surfaces were ground with abrasive to provide suitable traction for the beetles.

Additional pieces of glass (a) bonded to the inner walls of the entrance runway were used to focus the beetles to the center of the "Y". A baffle (b) was introduced to prevent premature mixing of the 2 air streams which gave the beetles a clearer choice. Openings allowed by the length of the baffle were of the minimum size required for passage of larger beetles. Confined beetles tend to defecate, creating a potential interfering

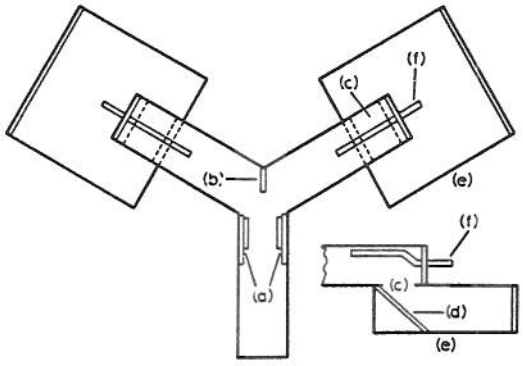

FIG. 1.—Customized "Y" olfactometer for *O. rhinoceros* (a) focusing plates, (b) baffle, (c) opening to holding chamber, (d) ramp, (e) holding chamber, and (f) air inlet tubes.

[1] Coleoptera: Scarabaeidae: Dynastinae.
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odor. This problem was solved by removing the beetles from the air flow after a choice was made. Openings (c) were constructed in the floor of the 2 choice arms of the olfactometer. Beetles would fall through these and slide down ramps (d) to the holding chambers (e). The air stream was carried past the openings of the choice arms by glass inlet tubes (f). Before each run, the doors of the holding chambers and the inlet tube assemblies were sealed with tape.

Fig. 2 indicates the air flow system. A small compressor pumped air in from a remote area outside the laboratory. The air was purified, humidified, passed through a flowmeter, and split into 2 streams for sample and control. A total flowrate of 4 liters/min gave the optimum response.

The necessity for continually introducing adults into the olfactometer meant that there was no effective way of removing the sample odor from the exit air. To protect the adults waiting to enter the olfactometer from possible overstimulation (Shorey 1970, Bartell and Lawrence 1973), they were placed in a plastic container with a stream of purified air passing continuously over them (Fig. 2).

All windows in the olfactometer room were blacked out. Minimal illumination was maintained with a single incandescent light located directly over the olfactometer. Room temperature and humidity were that of the natural environment (70°–90°; 75–85%).

The disastereoisomeric hydrocarbon mixture of (I) and (II) (2:1), prepared (Fig. 3) by polyphosphoric acid catalyzed cyclization of benzyloctins (III), was oxidized with chromium trioxide in aqueous acetic acid, and the resulting ketones, (IV) m.p. 72° and (V) m.p. 118°, were separated by chromatography on activated alkaline alumina in 22 and 30% overall yields following the method reported by Moore et al. (1970). Huang-Minlon

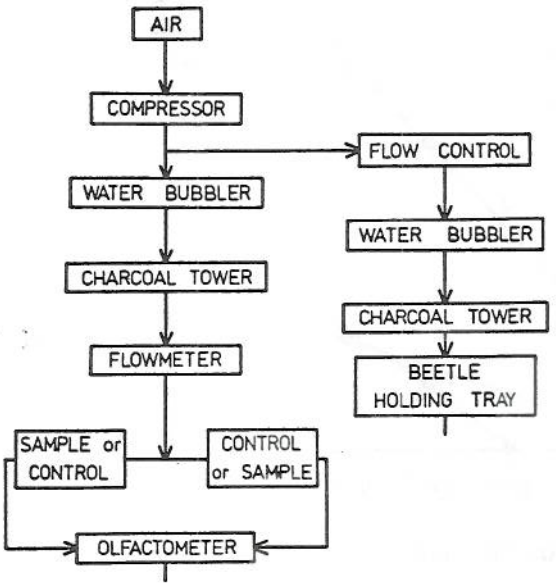

FIG. 2.—Olfactometer air flow system. (Note additional air flow over the beetle holding tray to prevent habituation.)

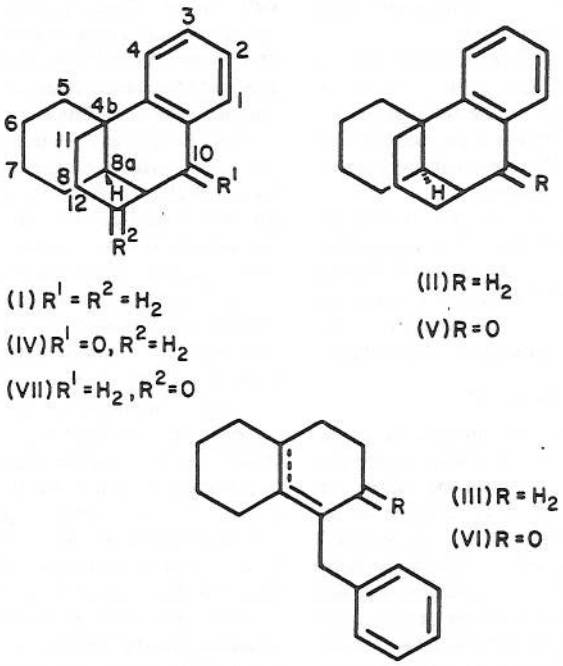

FIG. 3.—Synthetic routes for (±)-des-N-morphinan, I.

reduction of (IV) and (V) with 99% hydrazine hydrate, potassium hydroxide and diethylene glycol afforded the pure hydrocarbons (I) m.p. 57–58° and (II) (liquid). In a much simpler route cyclization of the benzyloctalone (VI) (Moore et al., 1970) with 85% orthophosphoric acid at 140–150°, for 4 h under a nitrogen atmosphere (minor modification of Stork 1968), gave the bridged-ketone (VII), m.p. 124–125° in 47% yield after column chromatography on activated alkaline alumina. Huang-Minlon reduction of this ketone afforded the hydrocarbon (I) in 84% yield.

Other test chemicals were obtained from the Entomology Research Division, Agricultural Research Service, USDA, except for rhinolure and geraniol which were obtained from the U.N./SPC Rhinoceros Beetle Projeet, Western Samoa, and Ajax Chemical, Ltd., respectively. Chemicals were applied as neat liquids or as diethyl ether solutions. In all cases, 0.01 ml of neat sample or ether solution was applied to a 2.0-cm filter paper disc to give a relatively constant sample area. The disc was put into an Erlenmeyer flask and topped with a drechsel bottle head, which was connected to the olfactometer and air flow system by polyethylene tubing.

Each cage of beetles was assigned an olfactometer. A complete trial consisted of 2 groups of 20–30 beetles put through their assigned olfactometers with the sample on one side, followed the next day with the sample on the opposite side. This procedure eliminated a bias inherent in the individual olfactometers and in the groups of beetles by adding day one bias to an equal and opposite bias on day 2. The results for the 2 groups were added together and analyzed statistically by chi-squared (χ^2) tests, assuming a null hypothesis of equal distribu-

tion and one degree of freedom. The beetles were used once a day between 9:00 and 11:00 a.m.

It required 4–6 min to put a group through their olfactometer. Longer runs were disregarded, as the remaining concentration of sample on the filter paper disc was more uncertain. The apparatus was cleaned with Decon 75 after each use, and all tubing that had come in contact with the chemical was replaced.

The response of *O. rhinoceros* to a series of 9 chemicals was tested with the olfactometer. The compounds (Table 1) had been previously field tested (Maddison 1972) and shown to be either not attractive or attractive in varying degrees. This series of chemicals provided direct comparison of field and laboratory experiments.

Results and Discussion

Table 1 shows a qualitative and quantitative correspondence between laboratory and field tests, especially important is the differentiation between the weaker attractants and powerful rhinolure. The variation in the percent response for the 2 runs of a trial is generally small, in particular this is true when a chemical elicits a definite positive response. These results indicate that the olfactory response of walking or flying beetles is not significantly different, and that the olfactometer can act as a good indicator of the field response of the beetles.

The 1st 3 chemicals in Table 1 had a χ^2 value corresponding to a probability of ca. $P = 0.05$ or less. These were chosen for further testing at different concentrations to determine how the beetle's response varied. Fig. 4 shows a clear linear relationship between the χ^2 value of the beetle's response and the concentration of the attractant used. These results give further confidence in the effectiveness of the olfactometer, and to the use of the simply calculated χ^2 value as a quantitative measure of an attractant's strength. Also, χ^2 plots allow a direct visual guide to the statistical significance of the data plotted. Direct plotting of the percent positive response versus concentration gave curved lines. Bartell and Shorey (1969) and Burkholder (1970) used probit analysis to obtain a linear relationship between response and attractant concentration.

The concentration studies provide information as to the lowest effective concentration of a chemical attractant as well as the rate at which the effectiveness falls off. These data can be useful and important in developing suitable trapping apparatus and in devising slow release systems for the attractant.

Fig. 5 shows the linear relationship between the attractant strength of I, in terms of χ^2, and its concentration. Attraction is still significant at the μg level ($\chi^2 = 3.84$; $P = 0.05$). These results are similar to those found for ethyl chrysanthemumate (rhinolure) the attractant currently used in field traps (Maddison et al. 1973). Although the 2 are of ca. equal effectiveness with respect to the amount placed on the filter paper discs, I has a much lower vapor pressure than rhinolure (0.008 ± 0.0002 nm and 0.30 ± 0.01 nm at 25°, respectively). Fewer molecules of I are required in the air stream to elicit the same response.

It has been well documented that insects are capable of distinguishing between geometrical (Roeloffs and Comeau 1971), and more recently, optical isomers (Borden et al. 1976). In marked contrast to I, diastereomer II had no effect on the beetles (10^{-3}g : $\chi^2 = (-)0.71 \pm 0.15$; 46%, n = 116). In addition to the different physical properties associated with diastereomers, the cis- trans-relationships of the substitutents at the 4b, 8a, and 9 carbons are altered due to the rigid ring system. The effect of the 2 enantiomers of I has not yet been investigated.

Although the structures of synthetic and natural attractants are quite diverse, they are generally characterized by having at least one oxygen containing functional

Table 1.—A comparison of *Oryctes rhinoceros* to the odor of 9 chemicals in olfactometer and field tests.

Chemical	Olfactometer results		Field results[c]
	%[a]	χ^2(n)[b]	
Ethyl chrysanthemumate (rhinolure)	70.2±0.6	15.4(94)	12
4-(*p*-Hydroxyphenyl)-2-butanone acetate (cue-lure)	60.0±0.0	4.0(100)	2
1-Methylproply trans-6-methyl-3-cyclohexane (siglure)	59.6±1.3	3.3(89)	4
2,5-Dimethyl-2,4-hexadiene	57.6±1.6	2.3(99)	4
Isopentyl butyrate	57.2±1.2	2.0(98)	2
Phenethyl butyrate	54.1±4.2	0.7(98)	1
Geraniol	52.0±2.0	0.2(100)	0[d]
Methyl eugenol	52.2±2.2	0.2(92)	0
trans-2-Hexenal	46.0±4.0	(−).7[e](94)	1

[a] Mean % response and range for the two beetle groups of each trial.
[b] $\chi^2 = 3.84$: $P = 0.05$; 6.64 : $P = 0.01$; 10.83 : $P = 0.001$
[c] Beetles captured/100 trap nights (Maddison 1973).
[d] Barber et al. (1971) reported captures of 1, 1, and 0 in three trails.
[e] Negative sign indicates a negative response. χ^2 is always positive.

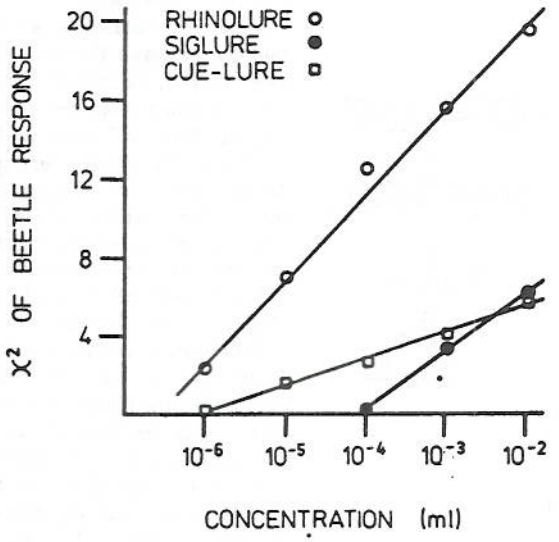

FIG. 4.—Concentration-activity relationships for 3 chemical attractants.

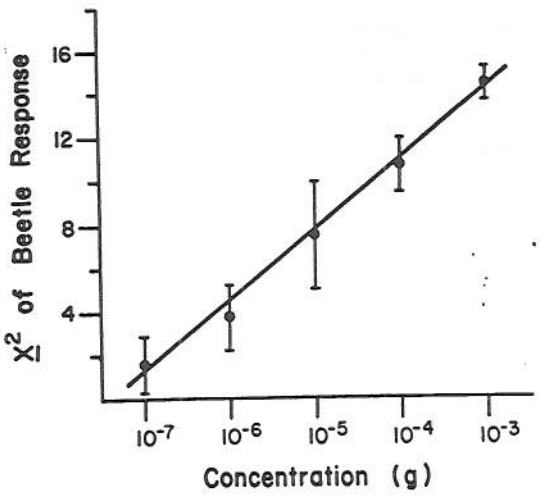

FIG. 5.—The concentration-activity relationship for the attraction of *O. rhinoceros* to (±)-des-N-morphinan (I). χ^2 = 3.84 : P = 0.05; χ^2 = 10.83 : P = 0.001. Percent beetles attracted at a concentration of 10^{-3} g = 68.0±0.1; 10^{-4} = 65.0±0.8; 10^{-5} = 62.5±2.2; 10^{-6} = 60.0±3; 10^{-7} = 55.0±2.8.

group. Simple alkanes, however, have been used by several species of formicine ants as alarm pheromones, (Blum 1975) and similarly monoterpene hydrocarbons, including α-pinene, are utilized as alarm substances by some termites (Moore 1975). To our knowledge, the only reported insect derived hydrocarbon attractants are 2-methylheptadecane, which has been found in several species of Arctiid moths (Roelofs and Cardé 1971) and (Z)-9-tricosene, a house fly attractant (Morgan et al. 1974). Although members of the *Dendroctonus* genus of bark beetles use host produced bicyclic monoterpene hydrocarbons as synergists for their own complex pheromone systems (Borden 1975), no other bridged hydrocarbon attractants have been reported. Compound I is both a highly effective and structurally unique chemical attractant, whose rigid geometry is ideally suited for determining structure-activity relationships.

The previously cited attraction of other insect species (Cherian 1974) to compound I, and the disruption of field tests in Western Samoa due to the remarkable attraction to the traps of skinks and geckoes (K. J. Marschall, pers. comm., 1976), hint at the broader scope and potential of this class of compounds. Further investigation of similar synthetic bridged hydrocarbons may yield other insect attractants, and shed light on structure-activity relationships.

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