Chamazulene from *Artemesia caruthii*

Sir:

In a recent survey of terpene-bearing plants of the Sonoran desert, it was reported (1) that the steam distillate of common sagebrush (*Artemesia caruthii* Wood var. *wrightii* A. Gray) contained a blue oil. Because of the widespread interest (2*a*, 2*b*) in the azulenes of the Compositae family, and in particular the Artemesia species, we undertook to identify the blue component of this oil. It has now been characterized as chamazulene (1,4-dimethyl-7-ethylazulene).

Stems, leaves, and flowers of *A. caruthii* were collected in August 1961 at elevations above 6000 ft. in the Graham Mountains of Arizona. The wet plant material was steam distilled; the blue distillate was taken up in petroleum ether and extracted into 85% phosphoric acid. The concentrated acid solution was diluted with water, regenerating the blue oil, which was then partitioned into petroleum ether again. After being dried over magnesium sulfate, the petroleum ether solution was evaporated to dryness under vacuum. A viscous blue oil remained; from 1.0 Kg. of wet plant material, 0.50 Gm. of azulenic compound was obtained.

After further purification by chromatography over alumina, the oil was converted to a trinitrobenzene addition compound; m.p. 130.5–131.0°, uncor. [Lit. (3), 132° for chamazulene TNB compound].

Anal.—Calcd. for $C_{14}H_{16}.C_6H_3(NO_2)_3$: C, 60.45; H, 4.82. Found: C, 60.35; H, 4.77.

The ultraviolet, visible, and infrared spectra were identical to the reported values (4) for chamazulene. As might be expected, these spectra were almost superimposable on those of an authentic sample of guaiazulene (1,4-dimethyl-7-isopropylazulene) measured in this laboratory. However, the critical data for the guaiazulene TNB compound [m.p. 151–152° (4); anal. calcd.: C, 61.31; H, 5.15] clearly differentiate it from the chamazulene compound.

Only a few plant species yield chamazulene on simple steam distillation. One of these is *Artemesia absynthium* (5). The precursors of this blue oil have been shown to be a group of closely-related sesquiterpene lactones (5). Because of the recent scientific interest in these sesquiterpene compounds, as well as local interest in the medicine and taxonomy of the Artemesia species in southwestern United States, we are now engaged in isolating and characterizing the lactones of *A. caruthii*.

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Screening Antifatigue Agents by Radiorespirometry

Sir:

Over the past 5 years a number of publications have appeared on the use of the potassium and magnesium salts of aspartic acid in the treatment of human fatigue (1–3). To measure the effect of various agents on fatigue in animals, the rat swim test has been found useful (4). Ideally, the mechanisms whereby these substances modify the energy-yielding processes in fatigue should be studied in the exercising animal. However, the many technical difficulties this would present led us to develop instead the following assay based on the utilization of glucose, a primary source of energy, during recovery from fatigue.

Male white rats, weighing 280 to 350 Gm. were burdened with lead amounting to 3% of their body weight and permitted to swim in tanks containing 18 inches of water at 28° until no longer able to surface.

Prior treatment consisted of 30 mg. per Kg. oral doses, beginning with a single dose 2 days before the experiment, two doses on the day before, and one or two on the day of the experiment, depending on whether the animal was to be used in the morning or afternoon, respectively. The last dose was given immediately before the animal was placed in the tank. Upon removal

TABLE I.—EFFECT OF ASPARTATE SALTS ON RESPIRATORY $C^{14}O_2$ PRODUCTION

Treatment	No. of Animals	Respired CO_2[a] 30 min.	60 min.	90 min.
Rested controls	5	2.18 ± 0.44	2.36 ± 0.62	1.75 ± 0.40
Swum controls	15	0.36 ± 0.15	0.62 ± 0.11	0.62 ± 0.25
K Asp. 30 mg./Kg.[b]	8	0.58 ± 0.32	0.96 ± 0.54	0.71 ± 0.25
P[c]		0.025	<0.025, >0.01	0.20
Mg Asp. 30 mg./Kg.	6	0.47 ± 0.32	0.66 ± 0.36	0.58 ± 0.22
P		0.15	0.35	...
K Asp. 15 mg./Kg. + Mg Asp. 15 mg./Kg.[d]	9	0.65 ± 0.40	0.91 ± 0.40	0.87 ± 0.18
P		0.05	<0.025, >0.01	<0.025, >0.01

[a] Means and standard deviations in $\mu c. \times 10^{-3}$ registered at the times indicated after injection of C^{14}-glucose. [b] Aspartate salts were given orally. [c] Significance of mean increase compared with swum controls by one-tailed *t*-test. [d] Spartase, Wyeth.

from the tank (discarding those which did not swim at least 15 minutes) 0.4 ml. of a solution containing 1.7 $\mu c.$ per ml. of uniformly labeled C^{14}-glucose in 2% nonradioactive glucose was injected into the external saphenous vein and the rat was placed in a closed glass chamber swept by 140 ml. of air per minute. The C^{14} content of the respired carbon dioxide was measured by passing the effluent air through the 250-ml. ionization chamber of a Nuclear-Chicago "Dynacon" electrometer connected to a 1 ma. linear recorder.

Representative data from the respired $C^{14}O_2$ curves for the controls and animals receiving individual and mixed aspartate salts are tabulated in Table I. The mean values for the control animals and those receiving the mixed salt are illustrated in Fig. 1, which demonstrates the essential factors observed in these experiments: (*a*) the length of the induction period before appreciable $C^{14}O_2$ is excreted (considerably prolonged in fatigued animals as compared with rested controls); (*b*) the considerably lower maximal rate of $C^{14}O_2$ evolution in the fatigued animals and the longer time taken to reach the maximal rate; (*c*) the differences in total $C^{14}O_2$ output as indicated by the areas under the curves; (*d*) the elevation of the curves of the treated animals above those of the fatigued controls.

Studies on the method are being continued in view of the fairly high degree of individual variation encountered, and glucose-1-C^{14} and glucose-6-C^{14} are being tried in the hope that one of these will result in a more specific response and also help to elucidate the mechanism.

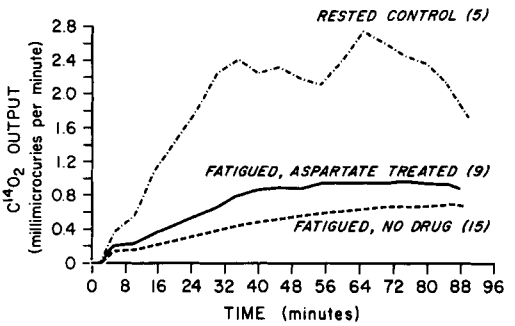

Fig. 1—Respiratory $C^{14}O_2$ output following C^{14}-glucose injection.

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l-N-Norarmepavine

Sir:

Recent reports have described the isolation from Asiatic lotus, *Nelumbo nucifera* Gaertn. (*Nymphaeaceae*), of the alkaloids nuciferine (1, 2, 4), roemerine (2, 4), nornuciferine (2–4), and *dl*-armepavine (5). We report herewith the isolation from American lotus, *Nelumbo lutea* (Willd.) Pers. (*Nymphaeaceae*)[1] of the alkaloids nuciferine (0.046%), *dl*-armepavine (0.0046%), and an apparently new alkaloid (0.047%), to which we assign structure I and the name *l*-N-norarmepavine.

The new alkaloid, m.p. 152–153°, $[\alpha]_D^{25}$ − 23°

[1] Air-dried leaves and stems, collected in Wisconsin in 1959–1961. We thank Professor H. H. Iltis of the University of Wisconsin for confirming the identity of the plant. A voucher specimen is deposited in the University of Wisconsin Herbarium.