References and Notes

1. Abbreviations: sRNA, soluble ribonucleic acid; tRNA, transfer ribonucleic acid, that is the fraction of sRNA which has the ability to accept and consequently transfer amino acids; DEAE, diethylaminoethyl; A, adenosine; Cp, 3′(2′)-cytidylic acid; Ap, 3′(2′)-adenylic acid; Gp, 3′(2′)-guanylic acid; Up, 3′(2′)-uridylic acid; ψp, 3′(2′)-pseudouridylic acid; 4-TUMP, 3′(2′),4-thiouridylic acid; pGp, 3′(2′),5′-guanosine diphosphate; pUp, 3′(2′),5′-uridine diphosphate; PPO, 2,5-diphenyloxazole; POPOP, *p*-bis [2-(5-phenyloxazolyl)]-benzene; BSA, bovine serum albumin; TCA, trichloroacetic acid.
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8. Radioassay procedures: The counting of ribonuclease-degraded RNA's labeled with S^{35} was done by taking 1- to 2-ml portions of column effluent and adding 20 ml of scintillation fluid of the following formula: 7 g PPO, 50 mg POPOP, and 70 g recrystallized napthalene per liter of *p*-dioxane (purified for spectroscopy). S^{35}-labeled RNA and H^3-uracil-labeled RNA were first precipitated by addition of 250 μg of BSA per milliliter of column effluent and then adding TCA to a final concentration of 5 percent. The precipitate was collected on a Whatman GF/A glass-filter pad, placed in a scintillation-counting vial, and dried at 90° for 30 minutes; this material was counted in 10 ml of scintillation fluid which contained: 4 g PPO and 50 mg POPOP per liter of redistilled toluene. Double-labeled fractions, S^{35}, H^3-uracil RNA from the countercurrent distribution phases, were counted in essentially the same way except that the two phases were first converted to a single phase by an ether extraction; the aqueous phase was then adjusted to *p*H 2 with 6*N* HCl, 400 μg BSA per milliliter was added first, then TCA to a final concentration of 10 percent. The precipitate was collected and handled as described for S^{35}-labeled RNA. The effluent fractions from Dowex-1 column chromatography were counted by taking 3 ml portions to dryness in a scintillation-counting vial and then adding 10 ml of the toluene-base scintillation-counting fluid. All counting was performed on a Nuclear Chicago 720 series liquid-scintillation counter.
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14. We thank Professor L. C. Craig, in whose laboratory this work was performed, for interest and support, and H. Lodish for advice and aid in the growth of bacterial cultures. Aided in part by PHS grant AM 02493-07.

28 September 1965

Flavonoids from the Moss Mnium affine Bland

Abstract. *Eight different flavone* C*-glycosides from* Mnium affine *were tentatively identified by chromatography and spectroscopy. Quercetin-3-diglycoside and other unidentified flavonoids were found in* M. arizonicum. *The flavonoid chemistry of* Mnium *is complex.*

Anthocyanin pigments in mosses have been reported (*1*), but the evidence has not been convincing until recently, when four anthocyanins from *Bryum* species were described (*2*); the pigments were reportedly 5-monoglucosides and 5-diglucosides of the rather uncommon anthocyanidins, apigeninidin and luteolinidin, which lack the 3-hydroxy substituent. Other types of flavonoids have not been reported from mosses, and the consensus has been that mosses are not a rich source of flavonoids.

We now report eight different flavone *C*-glycosides from the moss *Mnium affine*, additional flavone *C*-glycosides from *M. cuspidatum*, and the flavonol, quercetin-3-diglycoside, and other unidentified flavonoids in *M. arizonicum*. The flavonoid chemistry of *Mnium* species examined by us is comparable in complexity to that of angiosperms, and significant interspecific differences occur.

Gametophytic tissue of *M. affine* (*3*) was used to obtain two-dimensional chromatograms by methods described previously (*4*). Individual flavonoid compounds were eluted from replicate series of chromatograms and purified by repeated chromatography as necessary. Hydrolysis was effected by heating in 0.5*N* HCl in methanol for about 2 hours. Identification was confirmed by ultraviolet absorption, chromatographic data, and selected spray reagents.

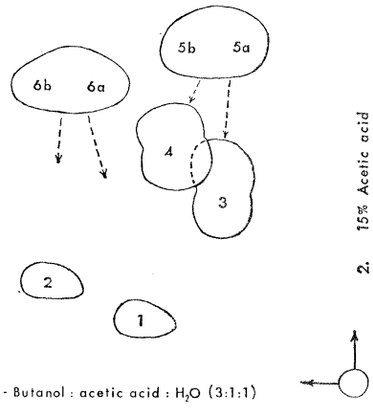

Fig. 1. Major *C*-glycosides in unhydrolyzed extracts of *Mnium affine*; arrows indicate the course of hydrolysis of specific flavonoids. [Drawn from chromatogram]

Compound 1 (Fig. 1, drawing of a two-dimensional chromatogram) runs at a position close to that of orientin (luteolin 8-*C*-glucoside) but yields ultraviolet spectra, obtained in methanol, sodium ethoxide, sodium acetate, and aluminum chloride (*5*), that although similar, are not identical with those of orientin.

The compound gives a negative result when the chromatogram is sprayed with Benedict's solution and observed in ultraviolet light, and thus it cannot have *o*-dihydroxy substituents —that is, it cannot be orientin. Scoparin, which is the 3′-methyl ether of orientin, has the chromatographic and spectral characteristics of compound 1. Compound 1 was therefore tentatively identified as scoparin, a *C*-glycoside (*6*).

Compound 2 (Fig. 1) is, by chromatography and absorption spectrum, identical with vitexin (apigenin-8-*C*-glycoside), a compound studied in the Lemnaceae (*7*) by these methods.

Compounds 3 and 4 may be chrysoeriol-6,8-di-*C*-glycoside and vicenin (apigenin-6,8-di-*C*-glycoside), respectively, on the basis of spectral and chromatographic data. Compound 3 is tentatively identified by analogy to the position and spectral features of lucenin; it gives a negative result by Benedict's test.

Compounds 1 to 4 resist acid hydrolysis. Compounds 5a and 5b partially overlap, are negative by Benedict's test, yield compounds 3 and 4 on acid hydrolysis, and are therefore considered to be *O*-glycosides of vicenin and the corresponding *C*-glycosidic derivative of chrysoeriol, respectively. Compounds 6a and 6b also partially overlap, are negative by Benedict's test, and yield compounds 1 and 2 on acid hydrolysis. Compounds 5a and 5b and 6a and 6b have not yet been separated; their spectra were otherwise clean, but had mixed characteristics.

We have little further information on the positions of attachment of the *O*-glycosides or on their identities. The 7-glucosides and 4′-glucosides of 6-*C*-glycosyl flavones are common, but the positions of the spots representing corresponding *O*-glycosides of 8-*C*-glycosyl flavones cannot be deduced from our present knowledge (*8*).

Related *C*-glycosides plus *O*-glyco-

sides were studied in another collection of *M. affine* and in *M. cuspidatum*. In the latter, saponaretin was identified as a hydrolytic derivative of a spot occupying the position of saponarin (7-*O*-glucoside of saponaretin). Diverse flavonoids including flavonol-3-glycosides have also been found in *M. arizonicum* (*8*).

Preliminary results indicate a rich flavonoid chemistry in *Mnium* that will contribute significantly to understanding of its systematics.

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Energy Intake of the Mouring Dove Zenaidura macroura marginella

Abstract. *Wild mourning doves follow a discrete cycle of feeding activity. Analysis of their food habits has provided an estimate for their average daily caloric intake.*

Samples of food taken from the crops of wild mourning doves during early fall of 1963 and 1964 provided evidence that the birds feed according to a discrete diurnal cycle. The data have been used to estimate daily caloric intake.

Crop samples were obtained from adult doves collected in Grand Forks County, North Dakota, between 15 August and 5 September. Birds were shot at various times of day and in various habitats in order to obtain a complete picture of feeding behavior.

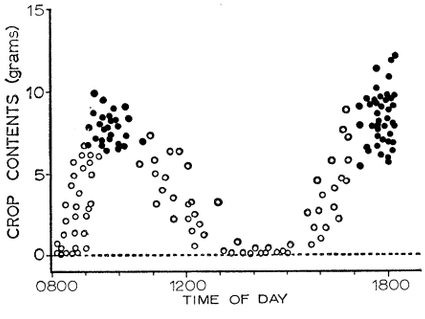

Fig. 1. Diurnal pattern of crop weight variation. The pattern of crop weights indicates two periods of feeding each day. The open circles (○) represent doves shot in fields or in roosting areas. The closed circles (●) represent doves with full crops, shot over water holes.

They were placed in plastic bags; labeled with the date, time of day, and habitat; and frozen for later analysis. After these specimens thawed, crop contents were removed, dried to constant weight at 100°C, and weighed. The contents of 120 crops were subsequently sorted according to seed species and reweighed. In addition, five samples of each of the nine major seed species were analyzed for caloric value by oxygen bomb calorimetry (Parr oxygen bomb calorimeter, Parr Instrument Co., Moline, Illinois).

Analysis of the food habits agreed with previous reports (*1*) which indicated that almost the entire diet of the mourning dove comes from plants. Nine seed species comprised 98.4 percent by weight of the 531.8 g of crop material examined (Table 1). An overall estimate of 4.41 kcal/g crop contents was obtained by weighting the caloric value of each seed species with the proportion of occurrence of each (by weight):

$$\sum_{i=1}^{9} (\text{kcal/g})_i \times (g_i/\text{total g}) = \text{kcal/g crop contents}$$

The daily variation in weight of crop contents indicated the pattern of feeding activity (Fig. 1). The birds fed twice each day, early in the morning and late in the afternoon. This observation is consistent with the suggestion of Davison and Sullivan (*2*). Examination of gizzards from birds with full crops, taken at water holes, indicated, since they lacked food materials, that digestion did not start until the crop contents were wetted. The daily pattern seemed to be: (i) early morning feeding to fill crops; (ii) drinking at water holes; (iii) roosting and digesting of food taken during the morning; (iv) late afternoon feeding to fill crops; and (v) evening drinking at water holes followed by flying to night roost areas.

Crops taken from the birds shot over water holes were used to estimate the total food intake of doves in a day. The average weight of seeds taken from crops after the morning feeding was $7.86 \pm .32$ ($SE_{\bar{x}}$) g, and after the evening feeding, $8.23 \pm .37$ g. The total weight of seed taken by a bird in a day was estimated to be the sum of that taken in the morning and in the evening periods, $16.09 \pm .49$ g/day. The daily caloric intake was obtained by multiplying the seed intake of 16.09 g/day by the caloric value of crop contents, 4.41 kcal/g, to yield an estimated average energy intake of 71 kcal/day per bird. A 95-percent confidence-interval estimate placed the daily energy intake per bird between 66.6 and 75.4 kcal.

Recognition of a discrete cycle of feeding activity in natural populations of the mourning dove provides an opportunity to analyze the energy budget

Table 1. Crop contents, with energy equivalents, of mourning doves in northeastern North Dakota.

Species of seed in crop	Occurrence (%)		Energy content (kcal/g)
	By bird*	By wt.†	
Green foxtail (*Setaria viridis*)	83.0	39.8	4.40
Yellow foxtail (*Setaria lutescens*)	68.9	21.4	4.70
Wheat (*Triticum aestivum*)	58.5	19.6	3.96
Black bindweed (*Polygonum convolvulus*)	25.5	5.1	4.21
Maize (*Zea mays*)	5.7	4.7	4.06
Proso millet (*Panicum miliaceum*)	3.8	3.0	4.29
Flax (*Linum usitatissimum*)	15.1	2.0	6.30
Field mustard (*Brassica arvensis*)	24.5	1.6	5.98
Lamb's quarters (*Chenopodium album*)	5.09	1.2	4.63

* Total number of crops = 120. † Total weight of crops is 531.8 g.